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Supplementary Materials for

Ultrasound imaging of gene expression in mammalian cells

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This PDF file includes:

Materials and Methods

Chemicals, cell lines and synthesized DNA

All chemicals were purchased from Sigma Aldrich unless otherwise noted. HEK293T and CHO-K1 cell lines were ordered from American Type Culture Collection (ATCC) and HEK293-tetON cells and CHO-tetON cells were purchased from Clontech (Takara Bio). Synthetic DNA was ordered from Twist Bioscience.

Cloning

Monocistronic plasmids used for transient transfection of HEK293T cells of gas vesicle genes used the pCMVSport backbone. Codon optimized gas vesicle genes were assembled in each plasmid using Gibson assembly. To test the effect of N- and C-terminal P2A modification each *B. megaterium* gas vesicle gene on the pNL29 plasmid (addgene 91696) was individually cloned using standard mutagenesis techniques. To test the N-terminal modification, the CCT codon was inserted following the start codon. To test the C-terminal modification, a linker-P2A sequence (GGAGCGCCAGGTTCCGGG-GCTACTAACTTCAGCCTCCTTAAACAGGCCGGCGA CGTGGAAGAGAATCCTGGC) was inserted upstream of the stop codon for each gene.

The polycistronic plasmid containing GvpN, GvpF, GvpG, GvpL, GvpS, GvpK, GvpJ, GvpU and Emerald GFP (EmGFP) were codon optimized, and synthesized in three fragments. The three fragments were Gibson assembled in the pCMVSport plasmid. The booster plasmid was assembled by multi-fragment Gibson assembly from PCR amplified fragments of the above plasmid.

The piggyBac transposon system (System Biosciences) was used to genomically integrate the mARG cassettes. To clone the mARG cassettes to the piggyBac transposon backbone, the plasmid was first digested using the SpeI and HpaI restriction enzymes and the mARG cassettes were Gibson assembled in the backbone. For doxycycline-inducible expression, the CMV promoter upstream of the gas vesicle genes was replaced with the TRE3G promoter. Internal ribosome entry site (IRES) and mCherry were cloned downstream GvpB as a marker for genomic integration. For the booster plasmid, CMVmin followed by enhanced BFP2 (eBFP2) and a polyadenylation element were cloned in the reverse direction upstream of the TRE3G promoter (creating a bi-directional doxycycline-inducible promoter) and used as a marker for genomic integration. A piggyBac transposon plasmid containing TRE3G and mCherry was Gibson assembled similarly to above.

Cell culture, transient transfection and TEM analysis

HEK293T and CHO-K1 cells were cultured in DMEM with 10% FBS and penicillin/streptomycin and seeded in a 6-well plate for transfection experiments. When the cells reached 70-80% confluency, 2 μg of total DNA (comprising the indicated mixtures of plasmids) was complexed with 2.58 μg polyethyleneimine (PEI-MAX; Polysciences Inc.) per μg of DNA, added to the cell culture, and incubated for 12-18 hours. The transfection of monocistronic plasmids encoding *Halobacterium salinarum*, *Anabaena flos-aquae* and *Bacillus megaterium* were all at equal molar ratios. Thereafter, the media containing the PEI-DNA complex was changed with fresh media. Cells were allowed to express the recombinant proteins for 72 hours.

To look for gas vesicles, fully confluent cells cultured in 6-well plates were lysed with 400 μL of Solulyse-M (Genlantis Inc) per well for one hour at 4 °C. The lysate was then transferred to 2 mL tubes, diluted with 800 μL of 10 mM HEPES buffer at pH 8.0 and centrifugated overnight at 300 g and 8 °C. Then, 60 μL of the supernatant was transferred to a fresh tube to be analyzed using transmission electron microscopy (TEM).

From this top fraction, 2 μL of sample was added to Formvar/carbon 200 mesh grids (Ted Pella) that were rendered hydrophilic by glow discharging (Emitek K100X). The samples were then stained with 2% uranyl acetate. The samples were imaged on a FEI Tecnai T12 transmission electron microscope equipped with a Gatan Ultrascan CCD.

To estimate gas vesicle yield and analyze size distribution, the cells were seeded in 6-well plates and gas vesicle expression was induced with 1 μg/mL of doxycycline and 5 mM sodium butyrate for 72 hours. The cells were lysed using Solulyse-M and buoyancy enriched at 300 g at 8 ˚C overnight. The top fraction of the supernatant was mixed with 2M urea and spotted on Formvar/carbon grids. The TEM grids were washed with water before staining with 2% uranyl acetate. To calculate gas vesicle yield per cell, the total number of gas vesicles per sub-grid on the TEM grid was manually counted and related via lysate volume to the number of source cells. Gas vesicle size distribution was quantified using FIJI (*30*).

To visualize gas vesicles inside cells, mARG-HEK cells were seeded in 6-well plates and allowed to express gas vesicles for 72 hours. The cells were fixed in 1.25% glutaraldehyde in PBS, post-fixed in 1% aqueous osmium tetroxide, reduced with ferrocyanide and block-stained in 1% uranyl acetate (all reagents from Electron Microscopy Sciences). The material was then dehydrated through a graded ethanol series and embedded in Eponate12 (Ted Pella). Sections were cut 60 nm thin onto formvar-filmed copper grids, stained with 2% uranyl acetate and Reynolds lead citrate, and imaged at 80 kV in a Zeiss EM10C (Oberkochen) equipped with an ES1000W Erlangshen CCD camera (Gatan).

Genomic integration and FACS

HEK293-tetON and CHO-tetON cells were used for genomic integration of the mARGs. The cells were cultured in a 6-well plate containing 2 mL DMEM with 10% tetracycline-free FBS (Clontech) and penicillin/streptomycin. Cells were transfected with the piggyBac transposon backbone containing the mARGs and the piggyBac transposase plasmid at a transposon:transposase molar ration of 2.5:1. Transfection was conducted using parameters mentioned above and the cells were allowed to incubate for 72 hours. Cells were induced with 1 μg/mL of doxycycline 24 hours prior to FACS (BD FACSAria III). Polyclonal subpopulations of mARG-expressing HEK293-tetON cells were sorted into the following four bins: (subtype 1) cells with eBFP2 fluorescence greater than 10^4 and EmGFP fluorescence greater than 10^4 and mCherry fluorescence greater than $2x10^4$ au, (subtype 2) cells with eBFP2 fluorescence between $3x10^3$ and $2x10^4$ and EmGFP fluorescence between $2x10^3$ and $2x10^4$ and mCherry fluorescence between $2x10^3$ and $2x10^4$ au, (subtype 3) cells with eBFP2 fluorescence between 10^3 and $6x10^3$ and EmGFP fluorescence between $2x10^2$ and 10^3 and mCherry fluorescence greater than $2x10^4$ au, (subtype 4) cells with eBFP2 fluorescence greater than 10^4 and EmGFP fluorescence greater than $2x10^4$ and mCherry fluorescence between $2x10^3$ and $2x10^4$ au. CHO-tetON cells were transfected with mARGs and the piggyBac transposase plasmid similar to above. mARG-expressing CHO-tetON cells with eBFP2 fluorescence greater than 10^4, EmGFP fluorescence greater than 10^4 and mCherry fluorescence greater than $2x10^4$ au were sorted.

For monoclonal cell lines, naïve HEK293-tetON cells were transfected with mARGs and the piggyBac transposase similar to above. mARG-expressing cells with eBFP2 fluorescence greater than 10^4, EmGFP fluorescence greater than 10^4 and mCherry fluorescence greater than $2x10^4$ au were sorted. 576 cells were sorted in individual wells of 96-well plate and the surviving 30 cells were analyzed for gas vesicle expression as described above.

To generate mCherry-HEK cells, HEK293-tetON cells were transfected with piggyBac transposon plasmid containing TRE3G promoter driving mCherry and the transposase plasmid similar to above. mCherry-HEK cells were sorted from cells with mCherry fluorescence between $1.5x10^4$ and 10^5 au.

Monoclonal cell lines (mARG-HEK and mCherry-HEK cells) were maintained in tetracycline-free media without butyrate and all imaging and toxicity experiments were conducted with cells that were less than 16 generations.

In vitro toxicity assays

The viability of the mARG-expressing cells was determined using three different assays involving cellular metabolic activity (resazurin reduction, MTT assay), quantification of cellular ATP content (CellTiter-Glo, Promega Corp.), and dye exclusion (Trypan Blue, Caisson Labs). The measurements were all quantified as percent viability compared to control cells that expressed mCherry only (mCherry-HEK). For the MTT and CellTiter-Glo assays, cells were grown in 96-well plates and induced with 1 µg/mL doxycycline and 5 mM sodium butyrate for 72 hours. They were then treated with reagents according the manufacturers' protocols. Luminescence (CellTiter-Glo) and absorbance at 540 nm (MTT) was measured using a SpectraMax M5 spectrophotometer (Molecular Devices). For the Trypan Blue assay, the cells were first grown in 6-well plates and treated with 1 µg/mL doxycycline and 5 mM sodium butyrate for 72 hours. They were then trypsinized and resuspended in media before being stained 1:1 with Trypan Blue dye. Ten µL of the solution was loaded in a disposable hemocytometer (C-chip DHC S02, Incyto) and total cell count and blue-stained dead cells were quantified by bright field microscopy. Cellular morphology was imaged from mARG-HEK and mCherry-HEK cells after 3 days of expression with 1 µg/mL doxycycline and 5 mM sodium butyrate. Phase images were acquired using a Zeiss Axio Observer with a 20x objective. For the co-culture cell competition assay, cells were counted and $2x10^5$ cells from each type were mixed together and seeded in 6-well plates. One day after seeding, cells were induced with 1 µg/mL doxycycline and 5 mM sodium butyrate and the media was exchanged daily. At each time point, cells were trypsinized and sorted using the MACSQuant VYB Flow Cytometer (Miltenyi Biotech) to quantify relative cell ratios. At one day and three days post induction, cells were passaged to ensure continuous exponential growth.

In vitro ultrasound imaging

To create phantoms for *in vitro* ultrasound imaging, wells were casted with molten 1% w/v agarose in PBS using a custom 3D-printed template. mARG-HEK and mCherry-HEK cells were allowed to express their transgenes using the specified inducer concentrations and expression duration. They were then trypsinized and counted via disposable hemocytometers in bright field microscopy. Next, cells were mixed at a 1:1 ratio with 50 °C agarose and loaded into the wells before solidification. The volume of each well was 60 µl and contained $6x10^6$ cells. The phantoms were submerged in PBS, and ultrasound images were acquired using a Verasonics Vantage programmable ultrasound scanning system and L22-14v 128-element linear array transducer with a 0.10-mm pitch, an 8-mm elevation focus, a 1.5-mm elevation aperture, and a center frequency of 18.5 MHz with 67% −6 dB bandwidth (Verasonics, Kirkland, WA). Each frame was formed from 89 focused beam ray lines, each with a 40-element aperture and 8 mm focus. A 3-half-cycle transmit waveform at 17.9 MHz was applied to each active array element. For each ray line, the amplitude modulation (AM) code was implemented using one transmit with all elements in the active aperture followed by 2 transmits in which first the odd- and then the even-numbered elements are silenced (*10*). Each image captured a circular cross-section of a well with a 4-mm diameter and center positioned at a depth of 8 mm. In AM mode, the signal was acquired at 0.27 MPa (2V) for 10 frames and the acoustic pressure was increased to 1.57 MPa (10V) to collect 46 additional frames. Ultrasound images were constructed by subtracting the collapsing frame by frame 4 post-collapse.

For Fig. 3, F-H, the high gas vesicle content of some samples resulted in acoustic shielding and a residual amount of gas vesicles remained intact after 46 frames of insonation at 1.57 MPa. To fully collapse all the gas vesicles and collect the background signal, the acoustic pressure was increased to 3.2 MPa (25V), then a second set of images was acquired with 10 frames at 0.27 MPa and 46 frames at 1.57 MPa. Gas vesicle-specific signal was determined by subtracting the total ultrasound signal from the 46 frames acquired before 3.2 MPa ultrasound by the total ultrasound signal from the 46 frames post collapse.

Cytotoxicity assay on cells exposed to ultrasound mARG-HEK and mCherry-HEK cells were cultured on custom made Mylar-bottom 24-well plates. Cells were cultured on fibronectin-coated Mylar films until they reached 80% confluency and induced for gas vesicle expression (1 µg/mL doxycycline and 5 mM sodium butyrate) for 3 days. The cells were then insonated from the bottom using an L22-14v 128-element linear array transducer (Verasonics). The transducer was mounted on a computer-controlled 3D translatable stage (Velmex). The bottom of the plates was acoustically coupled to the transducer with water and positioned 8 mm away from the transducer face. The cells were exposed to 3.2 MPa of pressure and the transducer was translated at a rate of 3.8 mm/s. The plates were returned to the incubator for 24 hours. Cytotoxicity was then assayed using resazurin reduction (MTT) on cells exposed to ultrasound and compared to non-insonated control cells.

3D cell culture and *in vitro* acoustic recovery after collapse

mARG-HEK and mCherry-HEK cells were mixed in Matrigel (Corning) containing 1 µg/mL of doxycycline and 5 mM sodium butyrate. The cell-laden hydrogels were placed in a 1% w/v agarose base to prevent cell migration out of the hydrogel and to separate the cells away from the bottom of the plates during imaging. Cells were cultured for total of 6 days and imaged every 3 days from the top using an L22-14v 128-element linear array transducer (Verasonics). The transducer was wiped with 70% ethanol, and imaging was conducted in a laminar flow biosafety cabinet to preserve sterility. After imaging, to ensure complete collapse of all gas vesicles in the cells, the entire hydrogel was exposed to 3.2 MPa ultrasound and the transducer was translated three times across the gel at a rate of 1-2 mm/s. The culture media was changed daily and contained 1 µg/mL of doxycycline and 5 mM sodium butyrate.

I*n vivo* expression of gas vesicles and ultrasound imaging

All *in vivo* experiments were performed on NOD SCID mice (NOD.CD17 *Prkdc^{scid}/NCrCr*l; Charles River), aged 10-15 weeks, under a protocol approved by the Institutional Animal Care and Use of Committee of the California Institute of Technology. mARG-HEK and mCherry-HEK cells were cultured in tetracycline-free media in T225 flasks. 1-1.2x10^7 cells were trypsinized and the 200 µl cell-pellet was mixed with 200 µl Matrigel (Corning) containing 5 mM sodium butyrate. The mixture of mARG-HEK cells and Matrigel was injected subcutaneously in the left flank of mice and the mixture of mCherry-HEK cells and Matrigel was injected subcutaneously in the right flank of mice. Starting from the day of tumor inoculation, mice we interperitoneally injected with 200 µl of saline containing 75 µg doxycycline and 25 mg of sodium butyrate daily. The lower half of mice were depilated to allow for fluorescence imaging and ultrasound coupling.

For ultrasound imaging, the mice were anesthetized with 2% isoflurane and maintained at 37˚C using a heating pad. Ultrasound imaging was carried out using the pulse sequence described above with an L22-14v transducer attached to a custom-made manual translation stage. Using B-mode ultrasound imaging, the center of the tumor was positioned approximately 8 mm from the surface of the transducer, and gas vesicle-specific ultrasound images were acquired. The transducer was translated laterally with 1 mm steps to collect ultrasound images of most of the tumor.

High framerate ultrasound datasets for Doppler imaging were acquired with the same ultrasound transducer and scanner. The Doppler pulse sequence consisted of 11 tilted plane wave transmissions (varying from -10 to 10 degrees) at a 5.5 kHz framerate, leading to a 500 Hz framerate after coherent compounding. Plane wave transmissions lasted 0.5 s (or 250 frames). A power Doppler image representing blood flow was computed from each ensemble of 250 frames using a singular value decomposition filter that separates clutter from red blood cell echoes (*31*).

To obtain tissue samples after the mice were euthanized, tumors were resected and placed in 3.7% formaldehyde solution (4˚C) for 24 hours and transferred to sterile 30% sucrose for an additional 24 hours. Tumors were embedded in OCT compound (Tissue-Tek), flash frozen and sectioned to 60 µm slices using a Cryostat (Leica CM3050). Sections were stained with TO-PRO3 nucleus stain, mounted (Fluoromount Aqueous Mounting Medium) and imaged using a Zeiss LSM 800 confocal microscope.

Supplementary Figures and Tables

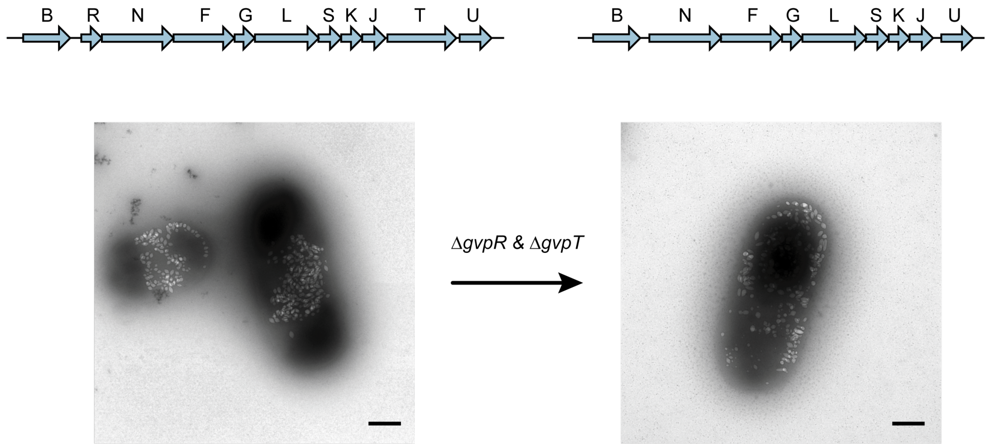


Fig. S1 – G*vpR* and G*vpT* genes in the *B. megaterium* gene cluster are not necessary for gas vesicle formation. Schematic of bacterial gas vesicle gene clusters used for heterologous expression of gas vesicles in *E. coli* (top). Representative whole cell TEM images of *E. coli* Rosetta 2(DE3)pLysS cells after expression of gas vesicles genes for 22 hours (bottom). Scale bars represent 500 nm. Expression performed as in Farhadi *et al.* (*21*) and TEM imaging as in Bourdeau *et al.* (*13*).

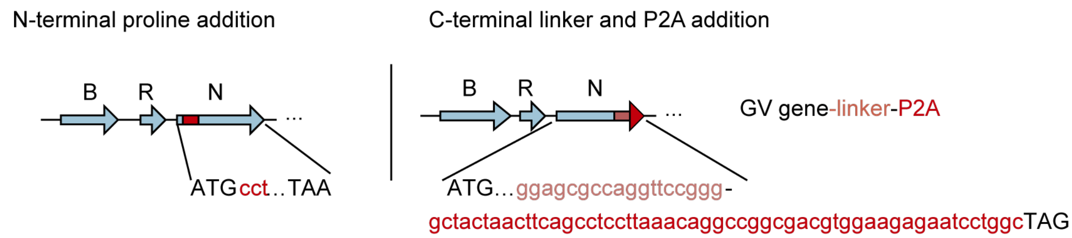


Fig. S2 – Assay for tolerability of P2A peptide additions. Illustration of gas vesicle gene cluster with N- or C-terminal modifications of each gene to test tolerability of P2A peptides, tested one-by-one in *E. coli*.

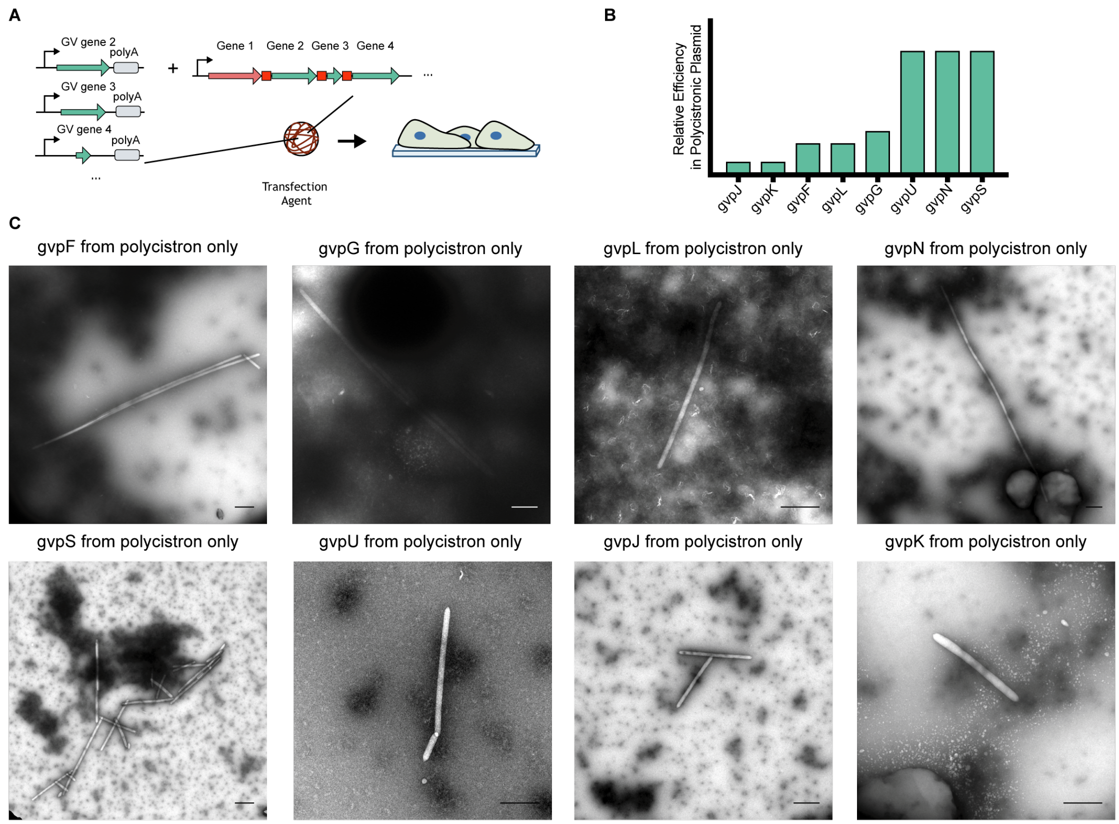
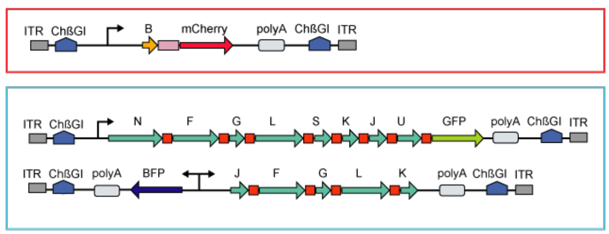


Fig. S3 – Identification of bottleneck genes on the polycistronic gas vesicle gene plasmid. (**A**) Schematic of the experiment. To test the efficiency with which gas vesicles could be formed when a given gene was supplied only on the polycistronic plasmid, and thereby identify "bottleneck" genes, cells were co-transfected with a monocistronic plasmid containing G*vpB*, 7 other monocistronic plasmids including all but the gene being assayed, and the polycistronic plasmid. (**B**) Qualitative estimate of the relative number of gas vesicles produced when each indicated gene was supplied solely by the polycistronic plasmid. (**C**) Representative TEM images of gas vesicles in the lysate of HEK293T cells for all 8 assays. Scale bars represent 500 nm. These results suggest that *GvpN*, *GvpS* and *GvpU* supplied in either monocistronic or polycistronic form supported abundant gas vesicle assembly. However, the production of gas vesicles was significantly reduced when *GvpJ, GvpF, GvpG, GvpL* or *GvpK* was supplied from the polycistronic vector. We therefore suspected that these genes represented a bottleneck in gas vesicle formation.

A



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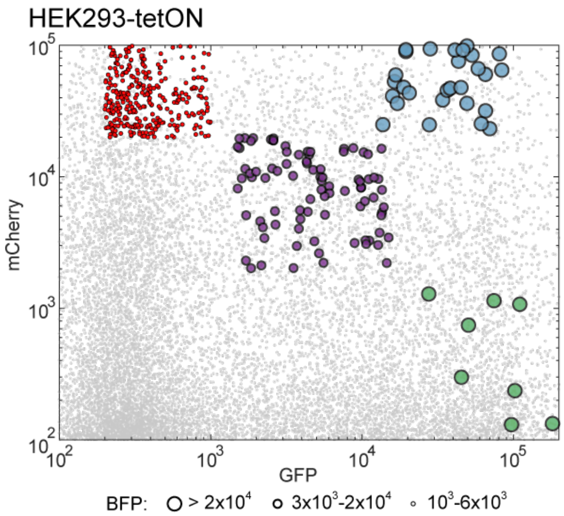

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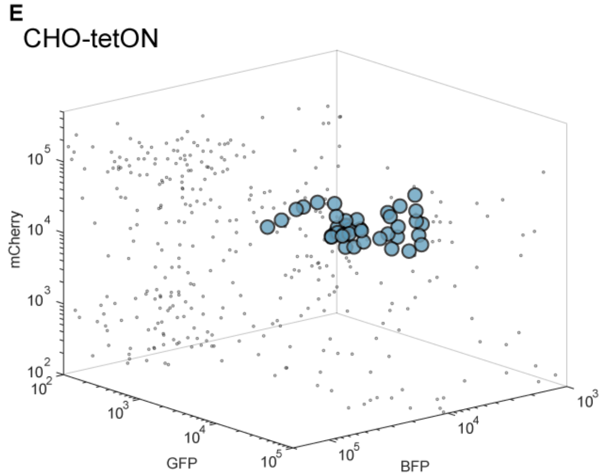

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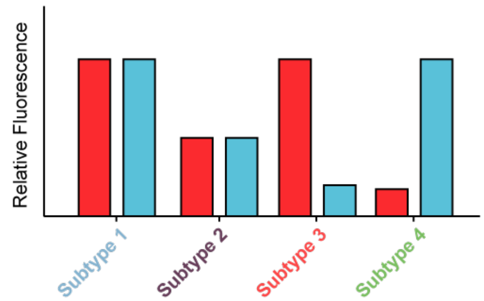
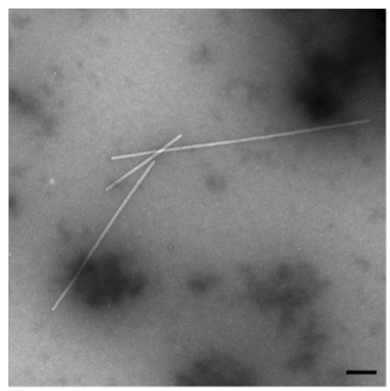


D

Subtype 1 (GV/cell)	Subtype 2 (GV/cell)	Subtype 3 (GV/cell)	Subtype 4 (GV/cell)
~0.25	~0.002	~0.001	~0.0005

F



G

Subtype 1 (GV/cell)
~0.1

Fig. S4 – Fluorescence activated cell sorting of HEK293-tetON and CHO-tetON cells transfected with integrating mARG constructs. (**A**) Diagram of the integrating constructs used to generate polyclonal cell lines. (**B**) FACS of mARG-expressing HEK293-tetON cells. Colored data indicate cells sorted for each group and gray dots are unsorted population. (**C**) Illustration of the four polyclonal subtypes sorted to study the impact of polycistron stoichiometry on gas vesicle expression. Red bars indicate mCherry expression; cyan bars indicate EmGFP and eBFP2 expression. (**D**) Approximate gas vesicle yield from polyclonal cells in each subtype. (**E**) FACS of mARG-expressing CHO-tetON cells. Colored data indicate cells sorted in subtype 1 and gray dots are unsorted cells. (**F**) Representative TEM image of buoyancy-enriched lysate from CHO-tetON cells sorted as indicated in (E). Scale bar represents 500 nm. (**G**) Approximate gas vesicle yield for the sorted mARG-expressing CHO-tetON cells.

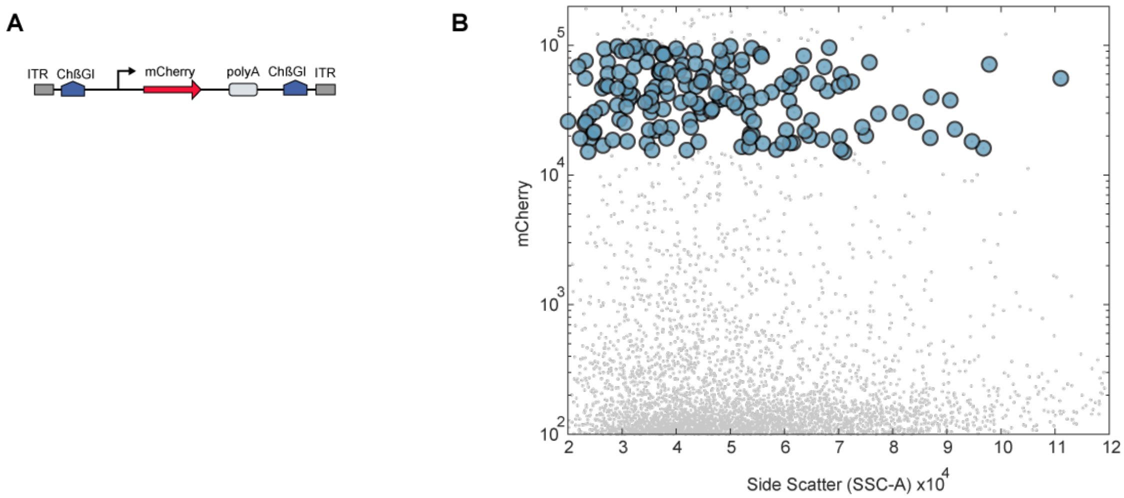


Fig. S5 – Genetic construct and sorting of mCherry-HEK cell line. (**A**) Genetic construct for stable genomic integration of mCherry containing a TRE3G promoter upstream and SV40 polyadenylation element downstream of mCherry. (**B**) FACS of mCherry cells, with selected cells indicated with blue dots.

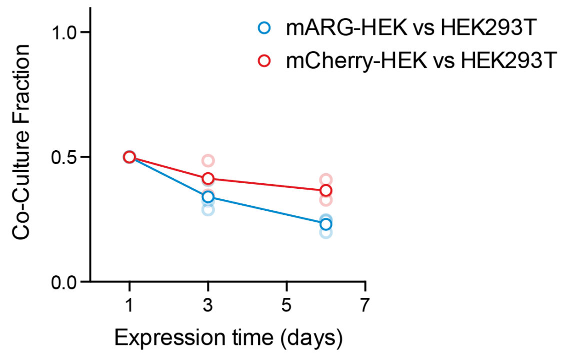


Fig. S6 – Co-culture of reporter gene expressing cells with HEK293T cells. Fraction of mARG-HEK cells in co-culture with HEK293T cells (blue) or mARG-mCherry cells in co-culture with HEK293T cells (red) seeded in equal numbers over 6 days of gene expression (n=3 biological replicates, each from 4 technical replicates, with darker dots showing the mean).

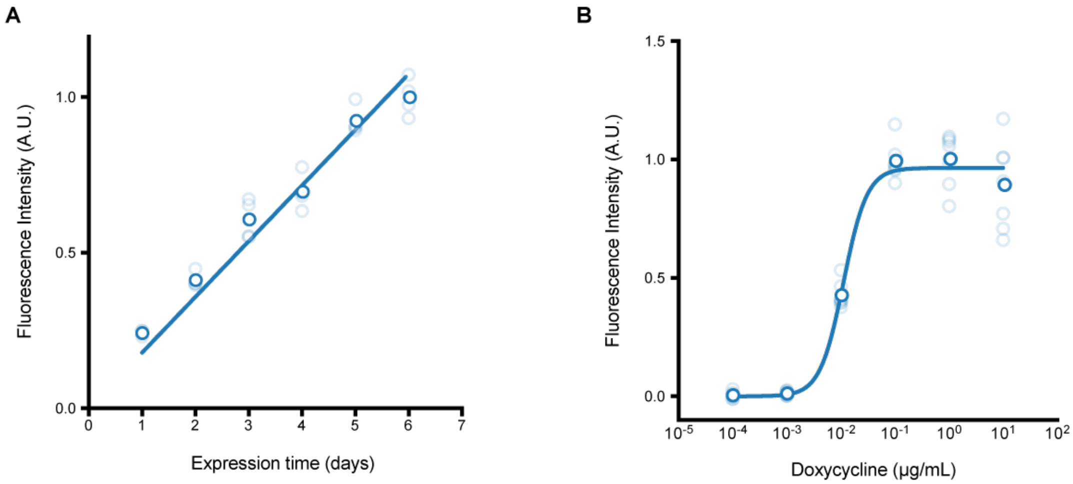


Fig. S7 – Fluorescence measurements of gene expression as a function of time and inducer concentration in mARG-HEK cells. (**A**) mCherry fluorescence of mARG-HEK cells induced with 1 µg/mL doxycycline and 5 mM sodium butyrate at the indicated times after induction (n=4, with the darker dots showing the mean). (**B**) mCherry fluorescence of mARG-HEK cells with the indicated inducer concentration and 5 mM sodium butyrate after 72 hours of induction (n=7, with the darker dots showing the mean).

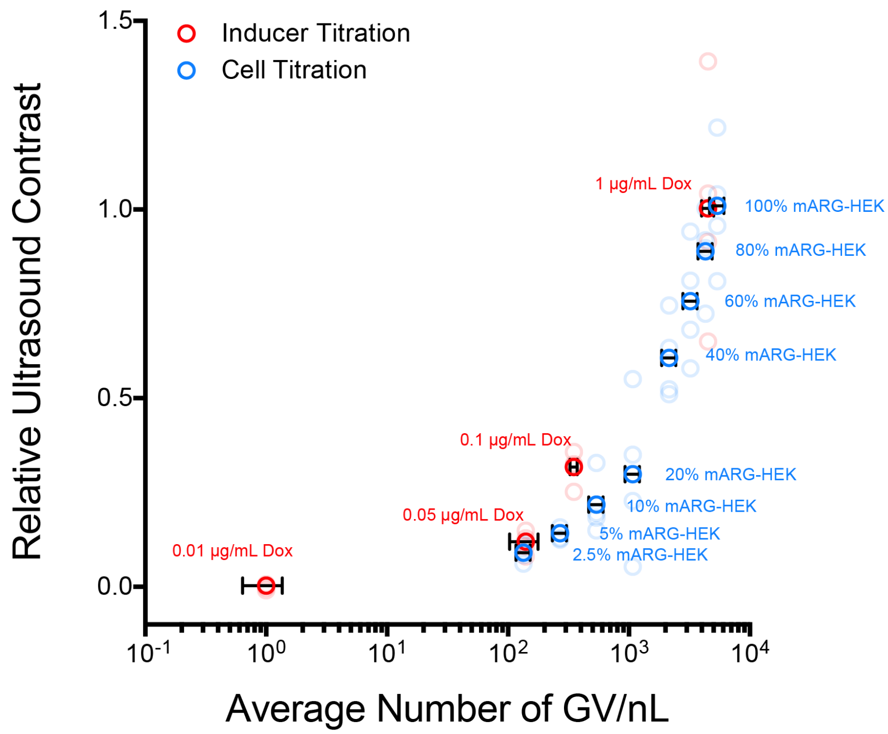


Fig. S8 – Dependence of ultrasound contrast on gas vesicle density. Relative ultrasound contrast produced by mARG-HEK cells in hydrogel as a function of the estimated average number of gas vesicles (GV) per nanoliter present after a monoculture of mARG-HEK cells was induced with different concentrations of doxycycline, or after fully-induced mARG-HEK cells were mixed with mCherry-HEK cells at different ratios. Blue symbols represent results from mARG-HEK cells induced with 1 µg/mL doxycycline for 3 days (producing on average 45 gas vesicles per cell) mixed with mCherry-HEK cells (expressing no gas vesicles) in varying proportions, as presented in Fig. 3H. Red symbols represent results from mARG-HEK cells induced with 0.01, 0.05, 0.1 and 1 µg/mL doxycycline for 3 days; expressing on average 0.01 ± 0.004, 1.4 ± 0.4, 3.5 ± 0.3, 45 ± 5.1 (mean ± SEM) gas vesicles per cell, respectively, as quantified by TEM. All cells were cultured with 5 mM sodium butyrate during expression. The number of gas vesicles was quantified after 72 hours of induced expression, as counted in lysates using TEM. Ultrasound contrast was normalized to the maximum in each type of titration. Dark symbols show the mean of ultrasound contrast for 4 replicates. Error bars represent SEM of 4 biological replicates for 0.01, 0.05, 0.1 µg/mL induction and n=3 biological replicates (each from two technical replicates) for 1 µg/mL samples.

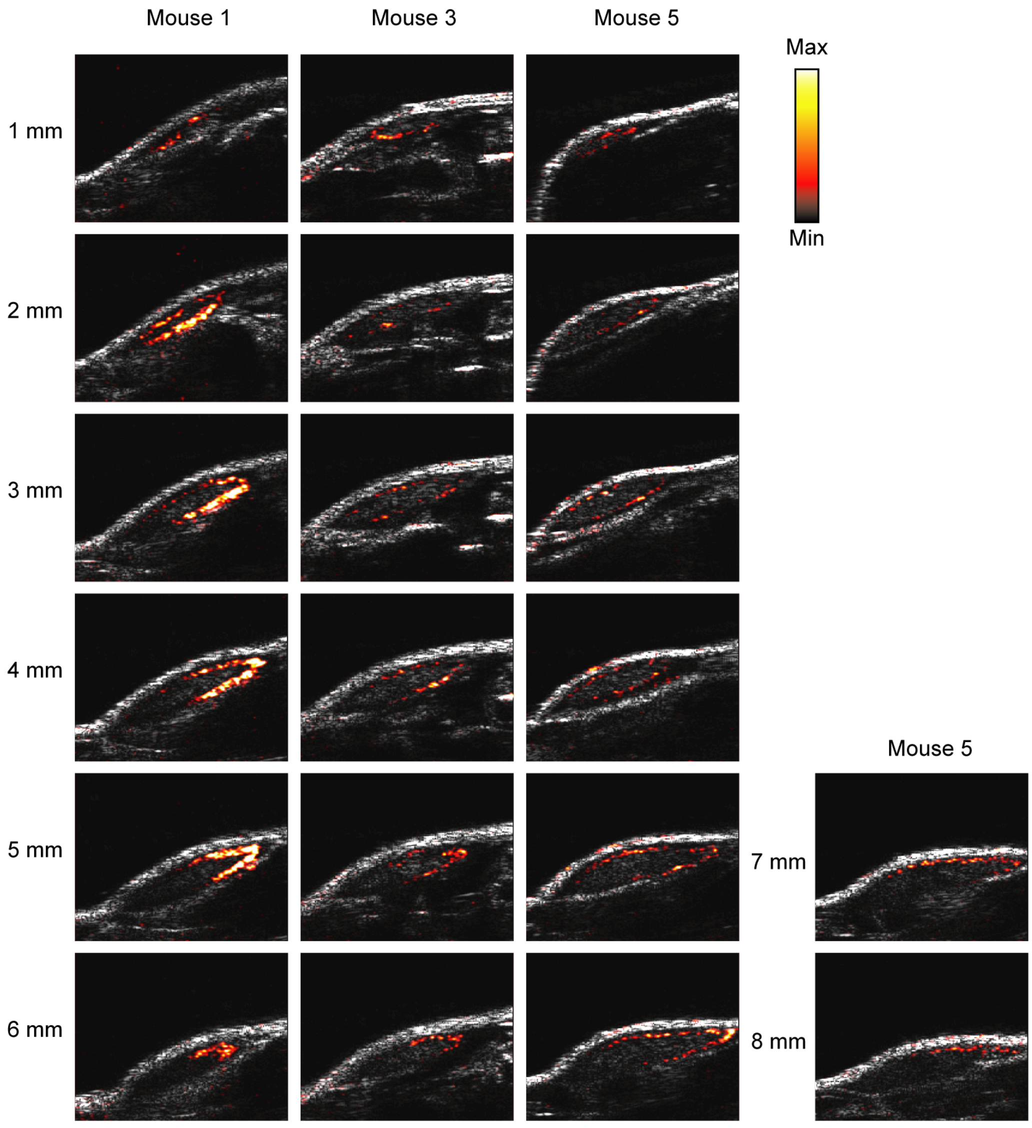


Fig. S9 – Additional examples of *in vivo* ultrasound images of adjacent planes in mARG-HEK tumors acquired at 1 mm intervals. For each imaging slice the difference heatmap of nonlinear signal between frame 1 and frame 4 is overlaid on grayscale anatomical scale. Minimum and maximum values of color bar are 4000 and 40000, respectively. Scale bars are 1 mm.

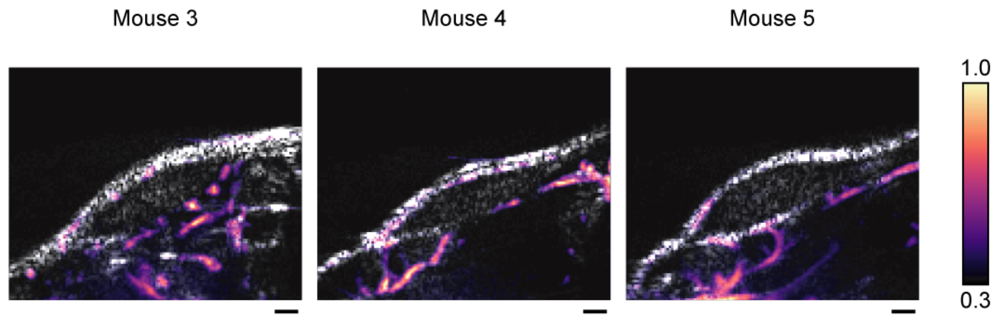


Fig. S10 – Representative Doppler ultrasound images of tumors containing mARG-HEK cells. Doppler ultrasound images were acquired using 250 frames of ultrafast planewaves at 25V and used to reconstruct vascular maps plotted as normalized power doppler signal overlaid on anatomical images in grayscale. Scale bars represent 1 mm.

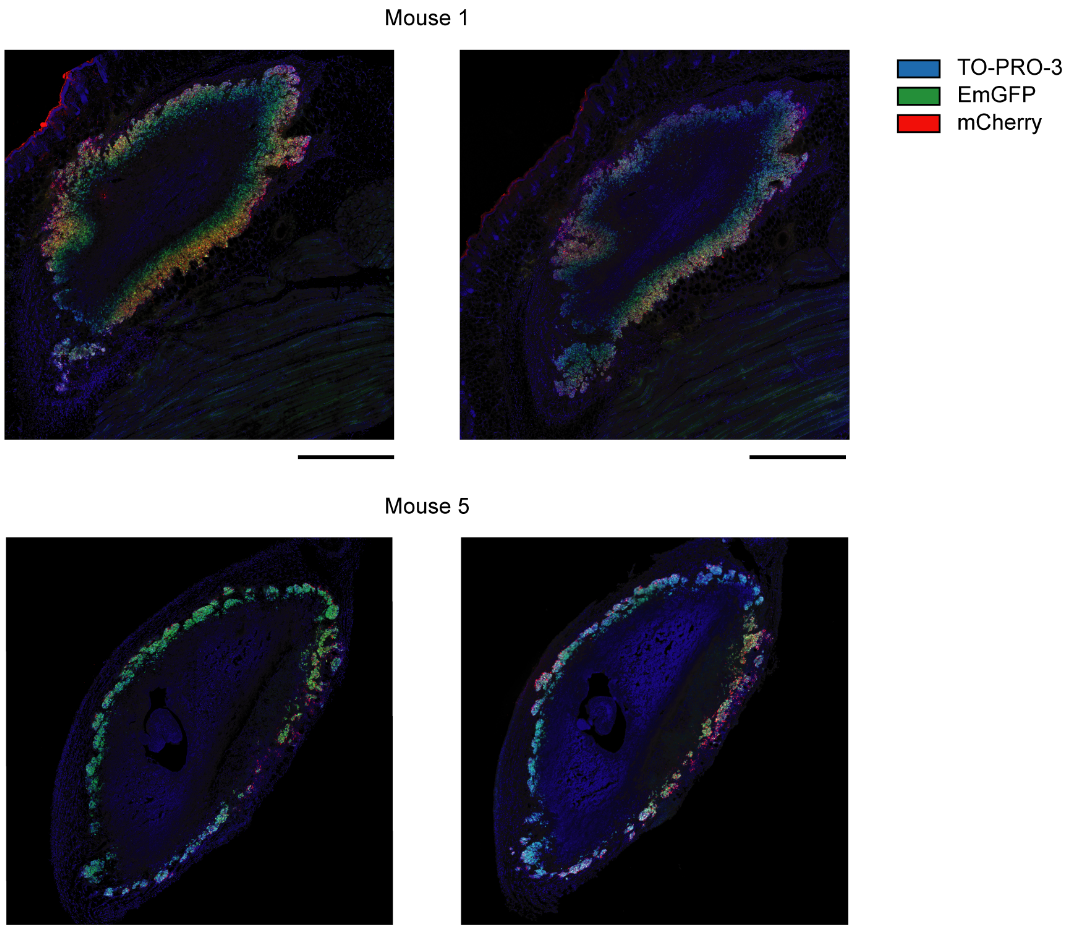


Fig. S11 – Representative histology sections of tumors containing mARG-HEK cells. For each mouse, two neighboring sections are presented. Blue color indicates cell nuclear staining using TO-PRO-3, green color represents GFP fluorescence and red color represents mCherry fluorescence. All scale bars are 1 mm.

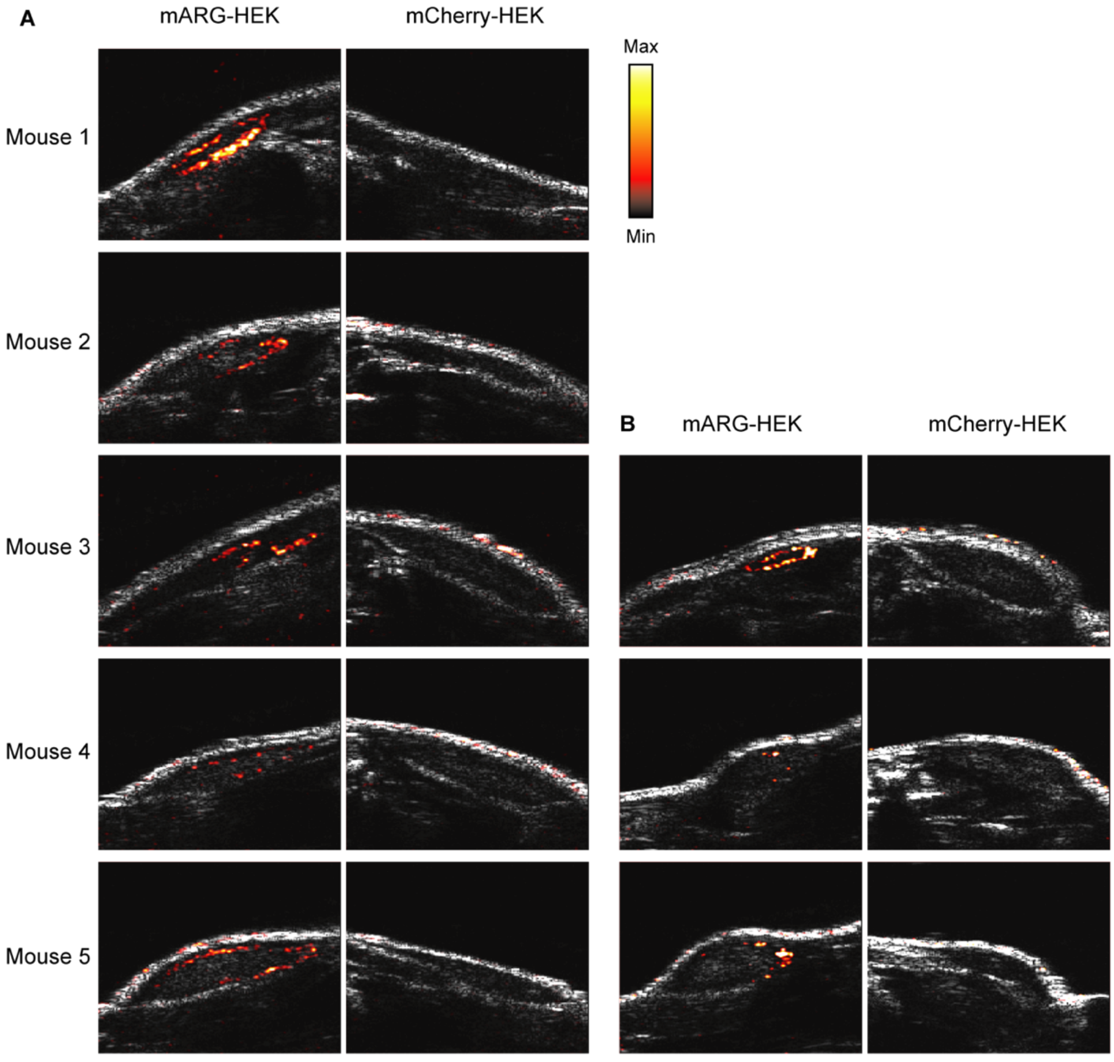


Fig. S12 – Biological replicates of *in vivo* ultrasound imaging of gene expression. (**A**) The left column shows ultrasound images of tumors containing mARG-HEK cells after 4 days of doxycycline administration. The right column shows ultrasound images of tumors containing mCherry-HEK cells after 4 days of doxycycline administration. After imaging the tumors were insonated with 3.2 MPa of ultrasound to collapse the expressed gas vesicles. (**B**) The left column shows ultrasound images of tumors containing mARG-HEK cells re-expressing gas vesicles after an additional 4 days of doxycycline administration. The right column shows ultrasound images of tumors containing mCherry-HEK cells after an additional 4 days of doxycycline administration. Difference heatmap of nonlinear signal between frame 1 and frame 4 is overlaid on a grayscale anatomical ultrasound image. Min and max on color bar represent 4000 and 40000, respectively. Scale bars represent 1 mm.

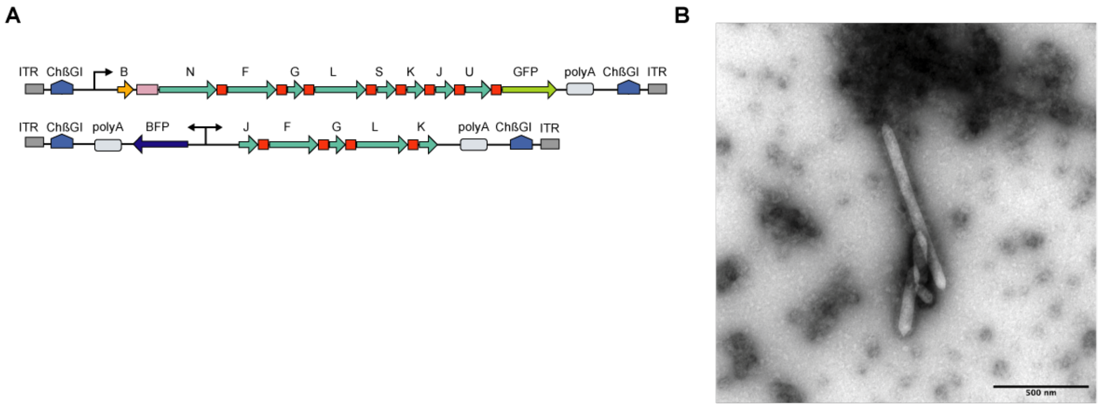


Fig. S13 – Consolidated mARG construct comprising 2 gene cassettes enables mammalian gas vesicle expression. (**A**) Schematic of two gene cassettes integrated to the genome of HEK293-tetON cells. In the top construct G*vpB* is separated from G*vpN* by an internal ribosome entry sequence (shown in purple). (**B**) Representative TEM image of GVs in the lysate of HEK293-tetON cells transfected with the constructs in (A) and induced with 1 μg/mL doxycycline.

Gene	GVs after N-term addition?	GVs after C-term addition?
GvpB	--	No
GvpR	Yes	Yes
GvpN	Yes	Yes
GvpF	Yes	Yes
GvpG	Yes	Yes
GvpL	Yes	Yes
GvpS	Yes	Yes
GvpK	Yes	Yes
GvpJ	Yes	Yes
GvpT	Yes	Yes
GvpU	Yes	Yes

Table S1 – Tolerability of P2A peptide additions to *B. megaterium* gas vesicle genes. Each gene of the *B. megaterium* gene cluster was modified with an N-terminal proline after the start codon or with a linker and P2A peptide at the C-terminus, resulting in a total of 21 unique GV gene clusters as illustrated in Fig. S2. *E. coli* were transformed with each plasmid and gas vesicles were induced for expression for a total of 22 hours and assayed for the presence of gas vesicles using TEM. The table indicates whether gas vesicles were observed by TEM. Expression and TEM imaging performed as in Farhadi *et al.* (*21*).

Collected from FACS	Formed colonies	Triple positive fluorescence	Formed GVs (TEM)	>1 GVs/cell
576	30	21	12	6

Table S2 – Selection funnel for monoclonal mARG-HEK cells. The numbers indicate the number of cells or cell lines selected at each stage.

DNA Sequences

GvpB	ATGAGCATCCAGAAGTCCACCAACAGCAGCAGCCTGGCCGAAGTGATCGACCGGATCCTGGACAA GGGCATCGTGATCGACGCCTTCGCCAGAGTGTCCGTCGTGGGCATCGAGATCCTGACCATCGAGG CCAGAGTCGTGATCGCCAGCGTGGACACCTGGCTGAGATATGCCGAAGCCGTGGGCCTGCTGCGG GACGACGTGGAAGAAAATGGCCTGCCCGAGCGGAGCAACAGCTCTGAGGGACAGCCCCGGTTCAG CATCTGA
GvpN	ATGACCGTGCTGACCGACAAGCGGAAGAAGGGCAGCGGCGCCTTCATCCAGGACGACGAGACAAA AGAGGTGCTGAGCAGAGCCCTGAGCTACCTGAAGTCCGGCTACAGCATCCACTTCACCGGACCTG CCGGCGGAGGCAAGACATCTCTGGCTAGAGCCCTGGCCAAGAAACGGAAGCGGCCCGTGATGCTG ATGCACGGCAACCACGAGCTGAACAACAAGGACCTGATCGGCGATTTCACCGGCTACACCAGCAA GAAAGTGATCGACCAGTACGTGCGGAGCGTGTACAAGAAAGACGAACAGGTGTCCGAGAACTGGC AGGACGGCAGACTGCTGGAAGCCGTGAAGAATGGCTACACCCTGATCTACGACGAGTTCACCAGA AGCAAGCCCGCTACCAACAACATCTTCCTGAGCATCCTGGAAGAGGGCGTGCTGCCCCTGTACGG CGTGAAGATGACCGACCCTTTCGTGCGCGTGCACCCCGACTTCAGAGTGATCTTCACCAGCAACC CCGCCGAGTATGCCGGCGTGTACGATACCCAGGACGCCCTGCTGGACCGGCTGATCACCATGTTC ATCGACTACAAGGACATCGACCGGGAAACCGCCATCCTGACCGAGAAAACCGACGTGGAAGAGGA CGAGGCCCGGACCATCGTGACCCTGGTGGCCAACGTGCGGAACAGAAGCGGCGACGAGAATAGCA GCGGCCTGAGCCTGAGAGCCAGCCTGATGATTGCCACCCTGGCCACCCAGCAGGACATCCCTATC GATGGCAGCGACGAGGACTTCCAGACCCTGTGCATCGACATCCTGCACCACCCCCTGACCAAGTG CCTGGACGAGGAAAACGCCAAGAGCAAGGCCGAGAAGATCATTCTGGAAGAGTGCAAGAACATCG ACACCGAGGAAAAGTGA
GvpF	ATGAGCGAGACAAACGAGACAGGCATCTACATCTTCAGCGCCATCCAGACCGACAAGGACGAGGA ATTCGGCGCCGTGGAAGTGGAAGGGACCAAGGCCGAGACATTCCTGATCCGGTACAAGGACGCCG CCATGGTGGCCGCCGAAGTGCCCATGAAGATCTACCACCCCAACCGGCAGAACCTGCTGATGCAC CAGAATGCCGTGGCCGCCATCATGGACAAGAACGACACCGTGATCCCCATCAGCTTCGGCAACGT GTTCAAGAGCAAAGAGGACGTGAAGGTGCTGCTGGAAAACCTGTACCCCCAGTTCGAGAAGCTGT TCCCCGCCATCAAGGGAAAGATCGAAGTGGGCCTGAAAGTGATCGGCAAGAAAGAGTGGCTGGAA AAGAAAGTGAACGAGAACCCCGAGCTGGAAAAAGTGTCCGCCAGCGTGAAGGGCAAGAGCGAGGC CGCTGGCTACTACGAGAGAATCCAGCTGGGCGGCATGGCCCAGAAGATGTTCACCAGCCTGCAGA AAGAAGTGAAAACCGACGTGTTCAGCCCCCTGGAAGAAGCCGCCGAGGCCGCCAAAGCCAATGAG CCTACAGGCGAGACAATGCTGCTGAACGCCAGCTTCCTGATCAACAGAGAGGACGAGGCCAAGTT CGACGAAAAAGTGAATGAGGCCCACGAGAACTGGAAGGATAAGGCCGACTTCCACTACAGCGGCC CCTGGCCCGCCTACAACTTCGTGAACATCCGGCTGAAGGTGGAAGAGAAGTGA
GvpG	ATGCTGCACAAGCTCGTGACCGCCCCCATCAACCTGGTCGTGAAGATCGGCGAGAAGGTGCAGGA AGAGGCCGACAAGCAGCTGTACGACCTGCCCACCATCCAGCAGAAGCTGATCCAGCTGCAGATGA TGTTCGAGCTGGGCGAGATCCCCGAGGAAGCCTTCCAGGAAAAAGAGGACGAGCTGCTGATGAGA TACGAGATCGCCAAGCGGCGCGAGATCGAGCAGTGGGAGGAACTGACCCAGAAGCGGAACGAGGA AAGCTGA
GvpL	ATGGGCGAGCTGCTGTACCTGTACGGCCTGATCCCCACCAAAGAGGCCGCTGCCATCGAGCCCTT CCCATTCTACAAGGGCTTCGACGGCGAGCACAGCCTGTACCCTATCGCCTTCGACCAAGTGACCG CCGTGGTGTTCAAGCTGGACGCCGACACCTACAGCGAGAAAGTGATCCAGGAAAAGATGGAACAG GACATGAGCTGGCTGCAGGAAAAGGCCTTCCACCACCACGAGACAGTGGCCGCCCTGTACGAGGA ATTCACCATCATCCCCCTGAAGTTCTGCACCATCTATAAGGGCGAGGAATCCCTGCAGGCCGCCA TCGAGATCAACAAAGAGAAGATCGAGAACTCCCTGACCCTGCTGCAGGGCAACGAGGAATGGAAC GTGAAGATCTACTGCGACGACACCGAGCTGAAGAAGGGCATCAGCGAGACAAACGAGAGCGTGAA GGCCAAGAAGCAGGAAATCAGCCACCTGAGCCCCGGCAGACAGTTCTTCGAGAAGAAGAAGATTG ACCAGCTGATCGAGAAAGAGCTGGAACTGCACAAGAACAAAGTGTGCGAGGAAATCCACGACAAG CTGATTGAGCTGAGCCTGTACGACTCCGTGAAGAAGAACTGGTCCAAGGACGTGACCGGCGCTGC CGAACAGATGGCCTGGAACAGCGTGTTCCTGCTGCCCAGCCTGCAGATCACCAAGTTCGTGAACG AGATCGAGGAACTGCAGCAGCGGCTGGAAAACAAGGGCTGGAAGTTCGAAGTGACCGGCCCCTGG CCTCCCTACCACTTCAGCAGCTTTGCCTGA
GvpS	ATGAGCCTGAAGCAGAGCATGGAAAACAAGGATATCGCCCTGATCGACATCCTGGACGTGATCCT GGACAAGGGCGTGGCCATCAAGGGCGACCTGATCATCTCTATCGCCGGCGTGGACCTGGTGTACC TGGACCTGAGAGTGCTGATCTCCAGCGTGGAAACCCTGGTGCAGGCCAAAGAGGGCAACCACAAG CCCATCACCAGCGAGCAGTTCGACAAGCAGAAAGAGGAACTGATGGACGCCACCGGCCAGCCCAG CAAGTGGACAAATCCTCTGGGCAGC

GvpK	ATGCAGCCCGTGTCCCAGGCCAACGGCAGAATCCACCTGGATCCCGATCAGGCCGAACAGGGACT GGCCCAGCTCGTGATGACCGTGATCGAGCTGCTGCGGCAGATCGTGGAACGGCACGCCATGAGAA GAGTGGAAGGCGGCACCCTGACCGACGAGCAGATCGAGAATCTGGGAATCGCCCTGATGAACCTG GAAGAGAAGATGGACGAGCTGAAAGAGGTGTTCGGACTGGACGCCGAGGACCTGAACATCGACCT GGGCCCTCTGGGCAGCCTGCTGTGA
GvpJ	ATGGCCGTGGAACACAACATGCAGAGCAGCACCATCGTGGACGTGCTGGAAAAGATCCTGGACAA GGGCGTCGTGATCGCCGGGGACATCACAGTGGGAATCGCCGACGTGGAACTGCTGACCATCAAGA TCCGGCTGATCGTGGCCAGCGTGGACAAGGCCAAAGAAATCGGCATGGATTGGTGGGAGAACGAC CCCTACCTGAGCAGCAAGGGCGCCAACAACAAGGCCCTGGAAGAGGAAAACAAGATGCTGCACGA GCGGCTGAAAACACTGGAAGAGAAGATCGAGACAAAGCGCTGA
GvpU	ATGAGCACCGGCCCCAGCTTCAGCACCAAGGACAACACCCTGGAATACTTCGTGAAGGCCAGCAA CAAGCACGGCTTCAGCCTGGACATCAGCCTGAACGTGAACGGGGCCGTGATCAGCGGCACCATGA TCAGCGCCAAAGAGTACTTCGACTACCTGAGCGAGACATTCGAAGAGGGCAGCGAGGTGGCCCAG GCCCTGTCTGAGCAGTTTAGCCTGGCCAGCGAGGCCTCCGAGTCTAATGGCGAAGCCGAGGCCCA CTTCATCCACCTGAAGAACACCAAGATCTACTGCGGCGACAGCAAGAGCACCCCCAGCAAGGGCA AGATCTTCTGGCGCGGCAAGATCGCCGAGGTGGACGGATTCTTCCTGGGAAAGATCAGCGACGCC AAGTCCACCAGCAAGAAGTCCAGCTGA

Table S3 – Sequences of nine *B. megaterium* genes used for gas vesicle expression in mammalian cells. All sequences are in the pCMVSport backbone with a CMV promoter upstream and SV40 polyA downstream each gene.

CMV:GvpNFGLSKJU-EmGFP:polyA	CGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGCCCATTGACGT CAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAG TATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTAT TGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTC CTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGTAC ATCAATGGGCGTGGATAGCGGTTTGACTCACGGGGATTTCCAAGTCTCCACCCCATTGACGTCAA TGGGAGTTTGTTTTGGCACCAAAATCAACGGGACTTTCCAAAATGTCGTAACAACTCCGCCCCAT TGACGCAAATGGGCGGTAGGCGTGTACGGTGGGAGGTCTATATAAGCAGAGCTCGTTTAGTGAAC CGTCAGATCGCCTGGAGACGCCATCCACGCTGTTTTGACCTCCATAGAAGACACCGGGACCGATC CAGCCTCCGGACTCTAGCCTAGGCTTTTGCAAAAAGCTATTTAGGTGACACTATAGAAGGTACGC CTGCAGGTACCGAGCTCGGATCCAGTACCCTTCACCATGACCGTGCTGACCGACAAGCGGAAGAA GGGCAGCGGCGCCTTCATCCAGGACGACGAGACAAAAGAGGTGCTGAGCAGAGCCCTGAGCTACC TGAAGTCCGGCTACAGCATCCACTTCACCGGACCTGCCGGCGGAGGCAAGACATCTCTGGCTAGA GCCCTGGCCAAGAAACGGAAGCGGCCCGTGATGCTGATGCACGGCAACCACGAGCTGAACAACAA GGACCTGATCGGCGATTTCACCGGCTACACCAGCAAAAAGGTGATCGACCAGTACGTGCGGAGCG TGTACAAGAAAGACGAACAGGTGTCCGAGAACTGGCAGGACGGCAGACTGCTGGAAGCCGTGAAG AATGGCTACACCCTGATCTACGACGAGTTCACCAGAAGCAAGCCCGCTACCAACAACATCTTCCT GAGCATCCTTGAGGAGGGCGTGCTGCCCCTGTACGGCGTGAAGATGACCGACCCTTTCGTGCGCG TGCACCCCGACTTCAGAGTGATCTTTACCAGCAACCCCGCCGAGTATGCCGGCGTGTACGATACC CAGGACGCCCTGCTGGACCGGCTGATCACCATGTTCATCGACTACAAGGACATCGACCGGGAAAC CGCTATCCTGACCGAGAAAACTGACGTGGAAGAAGACGAGGCCCGGACCATCGTGACCCTGGTGG CCAACGTGCGGAACAGAAGCGGCGACGAGAATAGCAGCGGCCTGAGCCTGAGAGCCAGCCTGATG ATTGCCACCCTGGCCACCCAGCAGGACATCCCTATCGATGGCAGCGACGAGGACTTCCAGACCCT GTGCATCGACATCCTGCACCACCCCCTGACCAAGTGCCTGGACGAAGAGAACGCCAAGAGCAAGG CCGAGAAGATCATTCTCGAAGAGTGCAAGAACATCGACACCGAGGAGAAGGGTGCCCCGGGATCT GGCGCAACAAATTTTAGTCTTTTAAAGCAGGCAGGAGACGTCGAGGAAAACCCTGGACCCGTGAG CGAGACAAACGAGACAGGCATCTACATCTTCAGCGCCATCCAGACAGACAAGGATGAGGAATTCG GCGCCGTGGAAGTGGAAGGGACCAAGGCTGAGACATTCCTGATCCGGTATAAGGACGCCGCCATG GTGGCCGCCGAAGTGCCCATGAAGATCTACCACCCCAACCGGCAGAACCTGCTGATGCACCAGAA TGCCGTGGCCGCCATCATGGACAAGAACGACACCGTGATCCCCATCAGCTTCGGCAACGTGTTCA AGAGCAAAGAGGACGTGAAGGTGCTCCTGGAAAACCTGTACCCCCAGTTCGAGAAGCTGTTCCCC GCCATCAAGGGAAAGATCGAAGTGGGCCTGAAGGTGATCGGCAAGAAAGAGTGGCTCGAAAAGAA AGTGAACGAGAACCCCGAGCTGGAAAAAGTGTCCGCCAGCGTGAAGGGCAAGAGCGAGGCCGCTG GCTACTACGAGAGAATCCAGCTGGGCGGCATGGCCCAGAAGATGTTCACAAGCCTGCAGAAAGAA GTGAAAACCGACGTGTTCAGCCCCCTGGAAGAAGCCGCCGAGGCCGCCAAAGCCAATGAGCCTAC AGGCGAAACAATGCTGCTGAACGCCAGCTTCCTGATCAACAGAGAGGATGAGGCCAAGTTCGACG AGAAAGTCAATGAGGCCCACGAGAACTGGAAGGATAAGGCCGACTTCCACTACAGCGGCCCCTGG CCCGCCTACAACTTCGTGAACATCCGGCTGAAGGTGGAAGAGAAGGGGGCACCTGGCTCGGGAGC GACCAACTTCTCATTACTCAAACAAGCCGGAGACGTTGAGGAGAATCCAGGCCCTGTGCTGCACA AGCTCGTGACCGCCCCCATCAACCTGGTCGTGAAGATCGGCGAGAAGGTGCAGGAAGAGGCCGAC AAGCAGCTGTACGACCTGCCCACCATCCAGCAGAAGCTGATCCAGCTGCAGATGATGTTCGAGCT GGGCGAGATCCCCGAGGAAGCCTTCCAGGAAAAAGAGGACGAACTGCTGATGAGATACGAGATCG CCAAGCGGCGCGAGATTGAGCAGTGGGAAGAACTGACCCAGAAGCGGAATGAGGAAAGCGGTGCC CCGGGATCTGGCGCAACAAATTTTAGTCTTTTAAAGCAGGCAGGAGACGTCGAGGAAAACCCTGG ACCCGTGGGCGAGCTGCTGTACCTCTACGGCCTGATCCCCACCAAAGAGGCCGCTGCTATCGAGC CCTTCCCATTCTACAAGGGCTTCGACGGCGAGCACAGCCTGTACCCTATCGCCTTCGACCAAGTG ACCGCCGTGGTGTTCAAGCTGGACGCCGACACCTACAGCGAGAAAGTGATCCAGGAAAAGATGGA ACAGGACATGAGCTGGCTGCAGGAAAAGGCCTTCCACCACCACGAGACAGTGGCCGCCCTGTATG AGGAATTCACCATCATCCCCCTGAAGTTCTGCACCATCTATAAGGGAGAGGAATCCCTGCAGGCC GCCATCGAGATCAACAAAGAGAAGATCGAAAACTCCCTGACCCTGCTGCAGGGCAACGAGGAATG GAACGTGAAGATCTACTGCGACGACACCGAGCTGAAGAAGGGCATCAGCGAGACAAACGAGAGCG TGAAGGCCAAGAAGCAGGAAATCAGCCACCTGAGCCCCGGCAGACAGTTCTTCGAGAAGAAGAAG ATTGACCAGCTCATCGAGAAAGAGCTGGAACTGCACAAGAACAAAGTGTGCGAGGAAATCCACGA CAAGCTGATTGAGCTGAGCCTCTACGACTCCGTGAAGAAGAACTGGTCCAAGGACGTGACAGGCG CTGCCGAACAGATGGCCTGGAACAGCGTGTTCCTGCTGCCCAGCCTGCAGATCACCAAGTTCGTG AACGAGATCGAGGAACTCCAGCAGCGGCTGGAGAACAAGGGATGGAAGTTCGAAGTGACCGGCCC CTGGCCTCCCTACCACTTCAGCAGCTTTGCCGGGGCACCTGGCTCGGGAGCGACCAACTTCTCAT

	TACTCAAACAAGCCGGAGACGTTGAGGAGAATCCAGGCCCTGTGAGCCTGAAGCAGAGCATGGAG AATAAGGATATCGCCCTGATCGACATCCTCGACGTGATCCTGGACAAGGGAGTGGCCATCAAGGG CGACCTGATCATCTCTATCGCCGGCGTGGACCTGGTGTACCTGGATCTGAGAGTGCTGATCTCCA GCGTGGAAACCCTGGTGCAGGCCAAAGAGGGCAACCACAAGCCCATCACCAGCGAGCAGTTCGAC AAGCAGAAAGAGGAGCTGATGGACGCCACCGGCCAGCCCAGCAAGTGGACAAATCCTCTGGGCAG CGGCGCTCCCGGGTCAGGTGCCACGAATTTTTCGTTGTTGAAGCAAGCTGGGGATGTTGAAGAGA ACCCAGGGCCTGTGCAGCCCGTGTCCCAGGCCAACGGCAGAATCCACCTGGATCCCGATCAGGCC GAACAGGGACTGGCCCAGCTCGTGATGACCGTGATCGAGCTGCTGCGGCAGATCGTGGAACGGCA CGCCATGAGAAGAGTGGAAGGCGGCACCCTGACCGACGAGCAGATCGAGAATCTGGGAATCGCTC TGATGAACCTGGAGGAGAAGATGGACGAGCTGAAAGAGGTGTTCGGACTGGACGCTGAGGATCTG AACATCGACCTGGGCCCTCTGGGCAGCCTGCTGGGTGCCCCGGGATCTGGCGCAACAAATTTTAG TCTTTTAAAGCAGGCAGGAGACGTCGAGGAAAACCCTGGACCCGTGGCCGTGGAACACAACATGC AGAGCAGCACCATCGTGGACGTGCTGGAAAAGATCCTGGACAAGGGCGTCGTGATCGCCGGGGAC ATCACAGTGGGAATCGCCGACGTGGAACTGCTGACCATCAAGATCCGGCTGATCGTGGCCAGCGT GGACAAGGCCAAAGAAATCGGCATGGATTGGTGGGAGAACGACCCCTACCTGAGCAGCAAGGGCG CCAACAACAAGGCTCTGGAAGAGGAAAACAAGATGCTGCACGAGCGGCTGAAAACACTGGAAGAG AAGATCGAGACAAAGCGCGGGGCACCTGGCTCGGGAGCGACCAACTTCTCATTACTCAAACAAGC CGGAGACGTTGAGGAGAATCCAGGCCCTGTGAGCACCGGCCCCAGCTTCAGCACCAAGGACAACA CCCTGGAATACTTCGTGAAGGCCAGCAACAAGCACGGCTTTAGCCTCGACATCAGCCTGAACGTG AATGGGGCCGTGATTAGCGGCACCATGATCAGCGCCAAAGAGTACTTCGACTACCTGAGCGAGAC ATTCGAAGAGGGCAGCGAAGTGGCCCAGGCCCTGTCTGAGCAGTTTAGCCTGGCTAGCGAGGCCT CCGAGTCTAATGGCGAAGCCGAGGCCCACTTCATCCACCTGAAGAACACCAAGATCTACTGCGGC GACAGCAAGAGCACCCCCAGCAAGGGCAAGATCTTCTGGCGCGGCAAGATCGCCGAGGTGGACGG ATTCTTCCTGGGAAAAATCAGCGACGCCAAGTCCACCAGCAAGAAGTCCAGCGGCGCTCCCGGGT CAGGTGCCACGAATTTTTCGTTGTTGAAGCAAGCTGGGGATGTTGAAGAGAACCCAGGGCCTGTG GTGTCCAAGGGCGAGGAACTGTTCACCGGCGTGGTGCCCATCCTGGTGGAACTGGATGGCGACGT GAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAAGGCGACGCCACATACGGAAAGCTGACCC TGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCTTGGCCTACCCTCGTGACCACACTGACC TACGGCGTGCAGTGCTTCGCCAGATACCCCGACCACATGAAGCAGCACGATTTCTTCAAGAGCGC CATGCCCGAGGGCTACGTGCAGGAACGGACCATCTTCTTCAAGGACGACGGCAACTACAAGACAA GAGCCGAAGTGAAGTTCGAGGGCGACACCCTCGTGAACCGGATCGAGCTGAAGGGCATCGACTTC AAAGAGGATGGCAACATCCTGGGCCACAAGCTGGAGTACAACTACAACAGCCACAAGGTGTACAT CACCGCCGACAAGCAGAAAAACGGCATCAAAGTGAACTTCAAGACCCGGCACAACATCGAGGACG GCAGCGTGCAGCTGGCCGACCACTACCAGCAGAACACCCCCATCGGAGATGGCCCCGTGCTGCTG CCCGACAACCACTACCTGAGCACACAAAGCGCCCTGAGCAAGGACCCCAACGAGAAGCGGGACCA CATGGTGCTGCTGGAATTTGTGACCGCCGCTGGCATCACCCTGGGCATGGACGAGCTGTACAAGT GACTCGAGTCTAGAGGGCCCCGTGGCTGTAATCTAGAGGATCCCTCGAGGGGCCCAAGCTTACGC GTGCATGCGACGTCATAGCTCTCTCCCTATAGTGAGTCGTATTATAAGCTAGCTTGGGATCTTTG TGAAGGAACCTTACTTCTGTGGTGTGACATAATTGGACAAACTACCTACAGAGATTTAAAGCTCT AAGGTAAATATAAAATTTTTAAGTGTATAATGTGTTAAACTAGCTGCATATGCTTGCTGCTTGAG AGTTTTGCTTACTGAGTATGATTTATGAAAATATTATACACAGGAGCTAGTGATTCTAATTGTTT GTGTATTTTAGATTCACAGTCCCAAGGCTCATTTCAGGCCCCTCAGTCCTCACAGTCTGTTCATG ATCATAATCAGCCATACCACATTTGTAGAGGTTTTACTTGCTTTAAAAAACCTCCCACACCTCCC CCTGAACCTGAAACATAAAATGAATGCAATTGTTGTTGTTAACTTGTTTATTGCAGCTTATAATG GTTACAAATAAAGCAATAGCATCACAAATTTCACAAATAAAGCATTTTTTTCACTGCATTCTAGT TGTGGTTTGTCCAAACTCATCAATGTATCTTATCATGTCTGGATC
CMV:GvpJ FGLK:poly A	CGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGCCCATTGACGT CAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAG TATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTAT TGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTC CTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGTAC ATCAATGGGCGTGGATAGCGGTTTGACTCACGGGGATTTCCAAGTCTCCACCCCATTGACGTCAA TGGGAGTTTGTTTTGGCACCAAAATCAACGGGACTTTCCAAAATGTCGTAACAACTCCGCCCCAT TGACGCAAATGGGCGGTAGGCGTGTACGGTGGGAGGTCTATATAAGCAGAGCTCGTTTAGTGAAC CGTCAGATCGCCTGGAGACGCCATCCACGCTGTTTTGACCTCCATAGAAGACACCGGGACCGATC CAGCCTCCGGACTCTAGCCTAGGCTTTTGCAAAAAGCTATTTAGGTGACACTATAGAAGGTACGC CTGCAGGTACCGAGCTCGGATCCAGTACCCTTCACCATGGCCGTGGAACACAACATGCAGAGCAG CACCATCGTGGACGTGCTGGAAAAGATCCTGGACAAGGGCGTCGTGATCGCCGGGGACATCACAG

	TGGGAATCGCCGACGTGGAACTGCTGACCATCAAGATCCGGCTGATCGTGGCCAGCGTGGACAAG GCCAAAGAAATCGGCATGGATTGGTGGGAGAACGACCCCTACCTGAGCAGCAAGGGCGCCAACAA CAAGGCCCTGGAAGAGGAAAACAAGATGCTGCACGAGCGGCTGAAAACACTGGAAGAGAAGATCG AGACAAAGCGCGGTGCCCCGGGATCTGGCGCAACAAATTTTAGTCTTTTAAAGCAGGCAGGAGAC GTCGAGGAAAACCCTGGACCCGTGAGCGAGACAAACGAGACAGGCATCTACATCTTCAGCGCCAT CCAGACAGACAAGGATGAGGAATTCGGCGCCGTGGAAGTGGAAGGGACCAAGGCTGAGACATTCC TGATCCGGTATAAGGACGCCGCCATGGTGGCCGCCGAAGTGCCCATGAAGATCTACCACCCCAAC CGGCAGAACCTGCTGATGCACCAGAATGCCGTGGCCGCCATCATGGACAAGAACGACACCGTGAT CCCCATCAGCTTCGGCAACGTGTTCAAGAGCAAAGAGGACGTGAAGGTGCTCCTGGAAAACCTGT ACCCCCAGTTCGAGAAGCTGTTCCCCGCCATCAAGGGAAAGATCGAAGTGGGCCTGAAGGTGATC GGCAAGAAAGAGTGGCTCGAAAAGAAAGTGAACGAGAACCCCGAGCTGGAAAAAGTGTCCGCCAG CGTGAAGGGCAAGAGCGAGGCCGCTGGCTACTACGAGAGAATCCAGCTGGGCGGCATGGCCCAGA AGATGTTCACAAGCCTGCAGAAAGAAGTGAAAACCGACGTGTTCAGCCCCCTGGAAGAAGCCGCC GAGGCCGCCAAAGCCAATGAGCCTACAGGCGAAACAATGCTGCTGAACGCCAGCTTCCTGATCAA CAGAGAGGATGAGGCCAAGTTCGACGAGAAAGTCAATGAGGCCCACGAGAACTGGAAGGATAAGG CCGACTTCCACTACAGCGGCCCCTGGCCCGCCTACAACTTCGTGAACATCCGGCTGAAGGTGGAA GAGAAGGGGGCACCTGGCTCGGGAGCGACCAACTTCTCATTACTCAAACAAGCCGGAGACGTTGA GGAGAATCCAGGCCCTGTGCTGCACAAGCTCGTGACCGCCCCCATCAACCTGGTCGTGAAGATCG GCGAGAAGGTGCAGGAAGAGGCCGACAAGCAGCTGTACGACCTGCCCACCATCCAGCAGAAGCTG ATCCAGCTGCAGATGATGTTCGAGCTGGGCGAGATCCCCGAGGAAGCCTTCCAGGAAAAAGAGGA CGAACTGCTGATGAGATACGAGATCGCCAAGCGGCGCGAGATTGAGCAGTGGGAAGAACTGACCC AGAAGCGGAATGAGGAAAGCGGTGCCCCGGGATCTGGCGCAACAAATTTTAGTCTTTTAAAGCAG GCAGGAGACGTCGAGGAAAACCCTGGACCCGTGGGCGAGCTGCTGTACCTCTACGGCCTGATCCC CACCAAAGAGGCCGCTGCTATCGAGCCCTTCCCATTCTACAAGGGCTTCGACGGCGAGCACAGCC TGTACCCTATCGCCTTCGACCAAGTGACCGCCGTGGTGTTCAAGCTGGACGCCGACACCTACAGC GAGAAAGTGATCCAGGAAAAGATGGAACAGGACATGAGCTGGCTGCAGGAAAAGGCCTTCCACCA CCACGAGACAGTGGCCGCCCTGTATGAGGAATTCACCATCATCCCCCTGAAGTTCTGCACCATCT ATAAGGGAGAGGAATCCCTGCAGGCCGCCATCGAGATCAACAAAGAGAAGATCGAAAACTCCCTG ACCCTGCTGCAGGGCAACGAGGAATGGAACGTGAAGATCTACTGCGACGACACCGAGCTGAAGAA GGGCATCAGCGAGACAAACGAGAGCGTGAAGGCCAAGAAGCAGGAAATCAGCCACCTGAGCCCCG GCAGACAGTTCTTCGAGAAGAAGAAGATTGACCAGCTCATCGAGAAAGAGCTGGAACTGCACAAG AACAAAGTGTGCGAGGAAATCCACGACAAGCTGATTGAGCTGAGCCTCTACGACTCCGTGAAGAA GAACTGGTCCAAGGACGTGACAGGCGCTGCCGAACAGATGGCCTGGAACAGCGTGTTCCTGCTGC CCAGCCTGCAGATCACCAAGTTCGTGAACGAGATCGAGGAACTCCAGCAGCGGCTGGAGAACAAG GGATGGAAGTTCGAAGTGACCGGCCCCTGGCCTCCCTACCACTTCAGCAGCTTTGCCGGGGCACC TGGCTCGGGAGCGACCAACTTCTCATTACTCAAACAAGCCGGAGACGTTGAGGAGAATCCAGGCC CTGTGCAGCCCGTGTCCCAGGCCAACGGCAGAATCCACCTGGATCCCGATCAGGCCGAACAGGGA CTGGCCCAGCTCGTGATGACCGTGATCGAGCTGCTGCGGCAGATCGTGGAACGGCACGCCATGAG AAGAGTGGAAGGCGGCACCCTGACCGACGAGCAGATCGAGAATCTGGGAATCGCCCTGATGAACC TGGAAGAGAAGATGGACGAGCTGAAAGAGGTGTTCGGACTGGACGCCGAGGACCTGAACATCGAC CTGGGCCCTCTGGGCAGCCTGCTGTGATAATCTAGAGGATCCCTCGAGGGGCCCAAGCTTACGCG TGCATGCGACGTCATAGCTCTCTCCCTATAGTGAGTCGTATTATAAGCTAGCTTGGGATCTTTGT GAAGGAACCTTACTTCTGTGGTGTGACATAATTGGACAAACTACCTACAGAGATTTAAAGCTCTA AGGTAAATATAAAATTTTTAAGTGTATAATGTGTTAAACTAGCTGCATATGCTTGCTGCTTGAGA GTTTTGCTTACTGAGTATGATTTATGAAAATATTATACACAGGAGCTAGTGATTCTAATTGTTTG TGTATTTTAGATTCACAGTCCCAAGGCTCATTTCAGGCCCCTCAGTCCTCACAGTCTGTTCATGA TCATAATCAGCCATACCACATTTGTAGAGGTTTTACTTGCTTTAAAAAACCTCCCACACCTCCCC CTGAACCTGAAACATAAAATGAATGCAATTGTTGTTGTTAACTTGTTTATTGCAGCTTATAATGG TTACAAATAAAGCAATAGCATCACAAATTTCACAAATAAAGCATTTTTTTCACTGCATTCTAGTT GTGGTTTGTCCAAACTCATCAATGTATCTTATCATGTCTGGATC

Table S4 – Transient polycistronic and booster plasmids in the pCMVSport backbone.

Piggybac transposon containing GvpB	ACTCTTCCTTTTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACATATT TGAATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCACCTAA ATTGTAAGCGTTAATATTTTGTTAAAATTCGCGTTAAATTTTTGTTAAATCAGCTCATTTTTTAAC CAATAGGCCGAAATCGGCAAAATCCCTTATAAATCAAAAGAATAGACCGAGATAGGGTTGAGTGTT GTTCCAGTTTGGAACAAGAGTCCACTATTAAAGAACGTGGACTCCAACGTCAAAGGGCGAAAAACC GTCTATCAGGGCGATGGCCCACTACGTGAACCATCACCCTAATCAAGTTTTTTGGGGTCGAGGTGC CGTAAAGCACTAAATCGGAACCCTAAAGGGAGCCCCCGATTTAGAGCTTGACGGGGAAAGCCGGCG AACGTGGCGAGAAAGGAAGGGAAGAAAGCGAAAGGAGCGGGCGCTAGGGCGCTGGCAAGTGTAGCG GTCACGCTGCGCGTAACCACCACACCCGCCGCGCTTAATGCGCCGCTACAGGGCGCGTCCCATTCG CCATTCAGGCTGCGCAACTGTTGGGAAGGGCGATCGGTGCGGGCCTCTTCGCTATTACGCCAGCTG GCGAAAGGGGGATGTGCTGCAAGGCGATTAAGTTGGGTAACGCCAGGGTTTTCCCAGTCACGACGT TGTAAAACGACGGCCAGTGAGCGCGCCTCGTTCATTCACGTTTTTGAACCCGTGGAGGACGGGCAG ACTCGCGGTGCAAATGTGTTTTACAGCGTGATGGAGCAGATGAAGATGCTCGACACGCTGCAGAAC ACGCAGCTAGATTAACCCTAGAAAGATAATCATATTGTGACGTACGTTAAAGATAATCATGTGTAA AATTGACGCATGTGTTTTATCGGTCTGTATATCGAGGTTTATTTATTAATTTGAATAGATATTAAG TTTTATTATATTTACACTTACATACTAATAATAAATTCAACAAACAATTTATTTATGTTTATTTAT TTATTAAAAAAAACAAAAACTCAAAATTTCTTCTATAAAGTAACAAAACTTTTATGAGGGACAGCC CCCCCCCAAAGCCCCCAGGGATGTAATTACGTCCCTCCCCCGCTAGGGGGCAGCAGCGAGCCGCCC GGGGCTCCGCTCCGGTCCGGCGCTCCCCCCGCATCCCCGAGCCGGCAGCGTGCGGGGACAGCCCGG GCACGGGGAAGGTGGCACGGGATCGCTTTCCTCTGAACGCTTCTCGCTGCTCTTTGAGCCTGCAGA CACCTGGGGGGATACGGGGAAAAGGCCTCCACGGCCACTAGTTTCACTCGAGTTTACTCCCTATCA GTGATAGAGAACGTATGAAGAGTTTACTCCCTATCAGTGATAGAGAACGTATGCAGACTTTACTCC CTATCAGTGATAGAGAACGTATAAGGAGTTTACTCCCTATCAGTGATAGAGAACGTATGACCAGTT TACTCCCTATCAGTGATAGAGAACGTATCTACAGTTTACTCCCTATCAGTGATAGAGAACGTATAT CCAGTTTACTCCCTATCAGTGATAGAGAACGTATGTCGAGGTAGGCGTGTACGGTGGGCGCCTATA AAAGCAGAGCTCGTTTAGTGAACCGTCAGATCGCCTGGAGCAATTCCACAACACTTTTGTCTTATA CTTGGTACCTATGCATGCCACCATGAGCATCCAGAAGTCCACCAACAGCAGCAGCCTGGCCGAAGT GATCGACCGGATCCTGGACAAGGGCATCGTGATCGACGCCTTCGCCAGAGTGTCCGTCGTGGGCAT CGAGATCCTGACCATCGAGGCCAGAGTCGTGATCGCCAGCGTGGACACCTGGCTGAGATATGCCGA AGCCGTGGGCCTGCTGCGGGACGACGTGGAAGAAAATGGCCTGCCCGAGCGGAGCAACAGCTCTGA GGGACAGCCCCGGTTCAGCATCTGAACTAAATCGCACTGTCGGCGTCCCCCCCTAACGTTACTGGC CGAAGCCGCTTGGAATAAGGCCGGTGTGCGTTTGTCTATATGTTATTTTCCACCATATTGCCGTCT TTTGGCAATGTGAGGGCCCGGAAACCTGGCCCTGTCTTCTTGACGAGCATTCCTAGGGGTCTTTCC CCTCTCGCCAAAGGAATGCAAGGTCTGTTGAATGTCGTGAAGGAAGCAGTTCCTCTGGAAGCTTCT TGAAGACAAACAACGTCTGTAGCGACCCTTTGCAGGCAGCGGAACCCCCCACCTGGCGACAGGTGC CTCTGCGGCCAAAAGCCACGTGTATAAGATACACCTGCAAAGGCGGCACAACCCCAGTGCCACGTT GTGAGTTGGATAGTTGTGGAAAGAGTCAAATGGCTCTCCTCAAGCGTATTCAACAAGGGGCTGAAG GATGCCCAGAAGGTACCCCATTGTATGGGATCTGATCTGGGGCCTCGGTGCACATGCTTTACATGT GTTTAGTCGAGGTTAAAAAACGTCTAGGCCCCCCGAACCACGGGGACGTGGTTTTCCTTTGAAAAA CACGATGATAATATGGCCACAACCATGGTGAGCAAGGGCGAGGAGGATAACATGGCCATCATCAAG GAGTTCATGCGCTTCAAGGTGCACATGGAGGGCTCCGTGAACGGCCACGAGTTCGAGATCGAGGGC GAGGGCGAGGGCCGCCCCTACGAGGGCACCCAGACCGCCAAGCTGAAGGTGACCAAGGGCGGCCCC CTGCCCTTCGCCTGGGACATCCTGTCCCCTCAGTTCATGTACGGCTCCAAGGCCTACGTGAAGCAC CCCGCCGACATCCCCGACTACTTGAAGCTGTCCTTCCCCGAGGGCTTCAAGTGGGAGCGCGTGATG AACTTCGAGGACGGCGGCGTGGTGACCGTGACCCAGGACTCCTCCCTGCAGGACGGCGAGTTCATC TACAAGGTGAAGCTGCGCGGCACCAACTTCCCCTCCGACGGCCCCGTAATGCAGAAGAAGACCATG GGCTGGGAGGCCTCCTCCGAGCGGATGTACCCCGAGGACGGCGCCCTGAAGGGCGAGATCAAGCAG AGGCTGAAGCTGAAGGACGGCGGCCACTACGACGCTGAGGTCAAGACCACCTACAAGGCCAAGAAG CCCGTGCAGCTGCCCGGCGCCTACAACGTCAACATCAAGTTGGACATCACCTCCCACAACGAGGAC TACACCATCGTGGAACAGTACGAACGCGCCGAGGGCCGCCACTCCACCGGCGGCATGGACGAGCTG TACAAGTGAACTAGTTCGTTAACTAAACTTGTTTATTGCAGCTTATAATGGTTACAAATAAAGCAA TAGCATCACAAATTTCACAAATAAAGCATTTTTTTCACTGCATTCTAGTTGTGGTTTGTCCAAACT CATCAATGTATCTTATCATGTCTGGAATTGACTCAAATGATGTCAATTAGTCTATCAGAAGCTCAT CTGGTCTCCCTTCCGGGGGACAAGACATCCCTGTTTAATATTTAAACAGCAGTGTTCCCAAACTGG GTTCTTATATCCCTTGCTCTGGTCAACCAGGTTGCAGGGTTTCCTGTCCTCACAGGAACGAAGTCC CTAAAGAAACAGTGGCAGCCAGGTTTAGCCCCGGAATTGACTGGATTCCTTTTTTAGGGCCCATTG GTATGGCTTTTTCCCCGTATCCCCCCAGGTGTCTGCAGGCTCAAAGAGCAGCGAGAAGCGTTCAGA GGAAAGCGATCCCGTGCCACCTTCCCCGTGCCCGGGCTGTCCCCGCACGCTGCCGGCTCGGGGATG

	CGGGGGGAGCGCCGGACCGGAGCGGAGCCCCGGGCGGCTCGCTGCTGCCCCCTAGCGGGGGAGGGA CGTAATTACATCCCTGGGGGCTTTGGGGGGGGGCTGTCCCTGATATCTATAACAAGAAAATATATA TATAATAAGTTATCACGTAAGTAGAACATGAAATAACAATATAATTATCGTATGAGTTAAATCTTA AAAGTCACGTAAAAGATAATCATGCGTCATTTTGACTCACGCGGTCGTTATAGTTCAAAATCAGTG ACACTTACCGCATTGACAAGCACGCCTCACGGGAGCTCCAAGCGGCGACTGAGATGTCCTAAATGC ACAGCGACGGATTCGCGCTATTTAGAAAGAGAGAGCAATATTTCAAGAATGCATGCGTCAATTTTA CGCAGACTATCTTTCTAGGGTTAATCTAGCTGCATCAGGATCATATCGTCGGGTCTTTTTTCCGGC TCAGTCATCGCCCAAGCTGGCGCTATCTGGGCATCGGGGAGGAAGAAGCCCGTGCCTTTTCCCGCG AGGTTGAAGCGGCATGGAAAGAGTTTGCCGAGGATGACTGCTGCTGCATTGACGTTGAGCGAAAAC GCACGTTTACCATGATGATTCGGGAAGGTGTGGCCATGCACGCCTTTAACGGTGAACTGTTCGTTC AGGCCACCTGGGATACCAGTTCGTCGCGGCTTTTCCGGACACAGTTCCGGATGGTCAGCCCGAAGC GCATCAGCAACCCGAACAATACCGGCGACAGCCGGAACTGCCGTGCCGGTGTGCAGATTAATGACA GCGGTGCGGCGCTGGGATATTACGTCAGCGAGGACGGGTATCCTGGCTGGATGCCGCAGAAATGGA CATGGATACCCCGTGAGTTACCCGGCGGGCGCGCTTGGCGTAATCATGGTCATAGCTGTTTCCTGT GTGAAATTGTTATCCGCTCACAATTCCACACAACATACGAGCCGGAAGCATAAAGTGTAAAGCCTG GGGTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCCGCTTTCCAGTCGGG AAACCTGTCGTGCCAGCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCGTATTGG GCGCTCTTCCGCTTCCTCGCTCACTGACTCGCTGCGCTCGGTCGTTCGGCTGCGGCGAGCGGTATC AGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGAACATGTG AGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCT CCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACT ATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCT TACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAG GTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCC CGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCC ACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTT GAAGTGGTGGCCTAACTACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCTCTGCTGAAGCC AGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGG TTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTT TTCTACGGGGTCTGACGCTCAGTGGAACGAAAACTCACGTTAAGGGATTTTGGTCATGAGATTATC AAAAAGGATCTTCACCTAGATCCTTTTAAATTAAAAATGAAGTTTTAAATCAATCTAAAGTATATA TGAGTAAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCT ATTTCGTTCATCCATAGTTGCCTGACTCCCCGTCGTGTAGATAACTACGATACGGGAGGGCTTACC ATCTGGCCCCAGTGCTGCAATGATACCGCGAGACCCACGCTCACCGGCTCCAGATTTATCAGCAAT AAACCAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTCCTGCAACTTTATCCGCCTCCATCCAGTC TATTAATTGTTGCCGGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTGCGCAACGTTGTTGC CATTGCTACAGGCATCGTGGTGTCACGCTCGTCGTTTGGTATGGCTTCATTCAGCTCCGGTTCCCA ACGATCAAGGCGAGTTACATGATCCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGTCCTCC GATCGTTGTCAGAAGTAAGTTGGCCGCAGTGTTATCACTCATGGTTATGGCAGCACTGCATAATTC TCTTACTGTCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTG AGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGATAATACCGCGCCACA TAGCAGAACTTTAAAAGTGCTCATCATTGGAAAACGTTCTTCGGGGCGAAAACTCTCAAGGATCTT ACCGCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGCACCCAACTGATCTTCAGCATCTTTTAC TTTCACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGC GACACGGAAATGTTGAATACTCAT
PiggyBac transposon containing GvpNFGLSKJU-EmGFP	ACTCTTCCTTTTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACATATT TGAATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCACCTAA ATTGTAAGCGTTAATATTTTGTTAAAATTCGCGTTAAATTTTTGTTAAATCAGCTCATTTTTTAAC CAATAGGCCGAAATCGGCAAAATCCCTTATAAATCAAAAGAATAGACCGAGATAGGGTTGAGTGTT GTTCCAGTTTGGAACAAGAGTCCACTATTAAAGAACGTGGACTCCAACGTCAAAGGGCGAAAAACC GTCTATCAGGGCGATGGCCCACTACGTGAACCATCACCCTAATCAAGTTTTTTGGGGTCGAGGTGC CGTAAAGCACTAAATCGGAACCCTAAAGGGAGCCCCCGATTTAGAGCTTGACGGGGAAAGCCGGCG AACGTGGCGAGAAAGGAAGGGAAGAAAGCGAAAGGAGCGGGCGCTAGGGCGCTGGCAAGTGTAGCG GTCACGCTGCGCGTAACCACCACACCCGCCGCGCTTAATGCGCCGCTACAGGGCGCGTCCCATTCG CCATTCAGGCTGCGCAACTGTTGGGAAGGGCGATCGGTGCGGGCCTCTTCGCTATTACGCCAGCTG GCGAAAGGGGGATGTGCTGCAAGGCGATTAAGTTGGGTAACGCCAGGGTTTTCCCAGTCACGACGT TGTAAAACGACGGCCAGTGAGCGCGCCTCGTTCATTCACGTTTTTGAACCCGTGGAGGACGGGCAG ACTCGCGGTGCAAATGTGTTTTACAGCGTGATGGAGCAGATGAAGATGCTCGACACGCTGCAGAAC

	ACGCAGCTAGATTAACCCTAGAAAGATAATCATATTGTGACGTACGTTAAAGATAATCATGTGTAA AATTGACGCATGTGTTTTATCGGTCTGTATATCGAGGTTTATTTATTAATTTGAATAGATATTAAG TTTTATTATATTTACACTTACATACTAATAATAAATTCAACAAACAATTTATTTATGTTTATTTAT TTATTAAAAAAAAACAAAAACTCAAAATTTCTTCTATAAAGTAACAAAACTTTTATGAGGGACAGCC CCCCCCCAAAGCCCCCAGGGATGTAATTACGTCCCTCCCCCGCTAGGGGGCAGCAGCGAGCCGCCC GGGGCTCCGCTCCGGTCCGGCGCTCCCCCCGCATCCCCGAGCCGGCAGCGTGCGGGGACAGCCCGG GCACGGGGAAGGTGGCACGGGATCGCTTTCCTCTGAACGCTTCTCGCTGCTCTTTGAGCCTGCAGA CACCTGGGGGGATACGGGGAAAAGGCCTCCACGGCCACTAGTTTCACTCGAGTTTACTCCCTATCA GTGATAGAGAACGTATGAAGAGTTTACTCCCTATCAGTGATAGAGAACGTATGCAGACTTTACTCC CTATCAGTGATAGAGAACGTATAAGGAGTTTACTCCCTATCAGTGATAGAGAACGTATGACCAGTT TACTCCCTATCAGTGATAGAGAACGTATCTACAGTTTACTCCCTATCAGTGATAGAGAACGTATAT CCAGTTTACTCCCTATCAGTGATAGAGAACGTATGTCGAGGTAGGCGTGTACGGTGGGCGCCTATA AAAGCAGAGCTCGTTTAGTGAACCGTCAGATCGCCTGGAGCAATTCCACAACACTTTTGTCTTATA CTTGGTACCTATGCATGCCACCATGACCGTGCTGACCGACAAGCGGAAGAAGGGCAGCGGCGCCTT CATCCAGGACGACGAGACAAAAGAGGTGCTGAGCAGAGCCCTGAGCTACCTGAAGTCCGGCTACAG CATCCACTTCACCGGACCTGCCGGCGGAGGCAAGACATCTCTGGCTAGAGCCCTGGCCAAGAAACG GAAGCGGCCCGTGATGCTGATGCACGGCAACCACGAGCTGAACAACAAGGACCTGATCGGCGATTT CACCGGCTACACCAGCAAAAAGGTGATCGACCAGTACGTGCGGAGCGTGTACAAGAAAGACGAACA GGTGTCCGAGAACTGGCAGGACGGCAGACTGCTGGAAGCCGTGAAGAATGGCTACACCCTGATCTA CGACGAGTTCACCAGAAGCAAGCCCGCTACCAACAACATCTTCCTGAGCATCCTTGAGGAGGGCGT GCTGCCCCTGTACGGCGTGAAGATGACCGACCCTTTCGTGCGCGTGCACCCCGACTTCAGAGTGAT CTTTACCAGCAACCCCGCCGAGTATGCCGGCGTGTACGATACCCAGGACGCCCTGCTGGACCGGCT GATCACCATGTTCATCGACTACAAGGACATCGACCGGGAAACCGCTATCCTGACCGAGAAAACTGA CGTGGAAGAAGACGAGGCCCGGACCATCGTGACCCTGGTGGCCAACGTGCGGAACAGAAGCGGCGA CGAGAATAGCAGCGGCCTGAGCCTGAGAGCCAGCCTGATGATTGCCACCCTGGCCACCCAGCAGGA CATCCCTATCGATGGCAGCGACGAGGACTTCCAGACCCTGTGCATCGACATCCTGCACCACCCCCT GACCAAGTGCCTGGACGAAGAGAACGCCAAGAGCAAGGCCGAGAAGATCATTCTCGAAGAGTGCAA GAACATCGACACCGAGGAGAAGGGTGCCCCGGGATCTGGCGCAACAAATTTTAGTCTTTTAAAGCA GGCAGGAGACGTCGAGGAAAACCCTGGACCCGTGAGCGAGACAAACGAGACAGGCATCTACATCTT CAGCGCCATCCAGACAGACAAGGATGAGGAATTCGGCGCCGTGGAAGTGGAAGGGACCAAGGCTGA GACATTCCTGATCCGGTATAAGGACGCCGCCATGGTGGCCGCCGAAGTGCCCATGAAGATCTACCA CCCCAACCGGCAGAACCTGCTGATGCACCAGAATGCCGTGGCCGCCATCATGGACAAGAACGACAC CGTGATCCCCATCAGCTTCGGCAACGTGTTCAAGAGCAAAGAGGACGTGAAGGTGCTCCTGGAAAA CCTGTACCCCCAGTTCGAGAAGCTGTTCCCCGCCATCAAGGGAAAGATCGAAGTGGGCCTGAAGGT GATCGGCAAGAAAGAGTGGCTCGAAAAGAAAGTGAACGAGAACCCCGAGCTGGAAAAAGTGTCCGC CAGCGTGAAGGGCAAGAGCGAGGCCGCTGGCTACTACGAGAGAATCCAGCTGGGCGGCATGGCCCA GAAGATGTTCACAAGCCTGCAGAAAGAAGTGAAAACCGACGTGTTCAGCCCCCTGGAAGAAGCCGC CGAGGCCGCCAAAGCCAATGAGCCTACAGGCGAAACAATGCTGCTGAACGCCAGCTTCCTGATCAA CAGAGAGGATGAGGCCAAGTTCGACGAGAAAGTCAATGAGGCCCACGAGAACTGGAAGGATAAGGC CGACTTCCACTACAGCGGCCCCTGGCCCGCCTACAACTTCGTGAACATCCGGCTGAAGGTGGAAGA GAAGGGGGCACCTGGCTCGGGAGCGACCAACTTCTCATTACTCAAACAAGCCGGAGACGTTGAGGA GAATCCAGGCCCTGTGCTGCACAAGCTCGTGACCGCCCCCATCAACCTGGTCGTGAAGATCGGCGA GAAGGTGCAGGAAGAGGCCGACAAGCAGCTGTACGACCTGCCCACCATCCAGCAGAAGCTGATCCA GCTGCAGATGATGTTCGAGCTGGGCGAGATCCCCGAGGAAGCCTTCCAGGAAAAAGAGGACGAACT GCTGATGAGATACGAGATCGCCAAGCGGCGCGAGATTGAGCAGTGGGAAGAACTGACCCAGAAGCG GAATGAGGAAAGCGGTGCCCCGGGATCTGGCGCAACAAATTTTAGTCTTTTAAAGCAGGCAGGAGA CGTCGAGGAAAACCCTGGACCCGTGGGCGAGCTGCTGTACCTCTACGGCCTGATCCCCACCAAAGA GGCCGCTGCTATCGAGCCCTTCCCATTCTACAAGGGCTTCGACGGCGAGCACAGCCTGTACCCTAT CGCCTTCGACCAAGTGACCGCCGTGGTGTTCAAGCTGGACGCCGACACCTACAGCGAGAAAGTGAT CCAGGAAAAGATGGAACAGGACATGAGCTGGCTGCAGGAAAAGGCCTTCCACCACCACGAGACAGT GGCCGCCCTGTATGAGGAATTCACCATCATCCCCCTGAAGTTCTGCACCATCTATAAGGGAGAGGA ATCCCTGCAGGCCGCCATCGAGATCAACAAAGAGAAGATCGAAAACTCCCTGACCCTGCTGCAGGG CAACGAGGAATGGAACGTGAAGATCTACTGCGACGACACCGAGCTGAAGAAGGGCATCAGCGAGAC AAACGAGAGCGTGAAGGCCAAGAAGCAGGAAATCAGCCACCTGAGCCCCGGCAGACAGTTCTTCGA GAAGAAGAAGATTGACCAGCTCATCGAGAAAGAGCTGGAACTGCACAAGAACAAAGTGTGCGAGGA AATCCACGACAAGCTGATTGAGCTGAGCCTCTACGACTCCGTGAAGAAGAACTGGTCCAAGGACGT GACAGGCGCTGCCGAACAGATGGCCTGGAACAGCGTGTTCCTGCTGCCCAGCCTGCAGATCACCAA GTTCGTGAACGAGATCGAGGAACTCCAGCAGCGGCTGGAGAACAAGGGATGGAAGTTCGAAGTGAC

	CGGCCCCTGGCCTCCCTACCACTTCAGCAGCTTTGCCGGGGCACCTGGCTCGGGAGCGACCAACTT CTCATTACTCAAACAAGCCGGAGACGTTGAGGAGAATCCAGGCCCTGTGAGCCTGAAGCAGAGCAT GGAGAATAAGGATATCGCCCTGATCGACATCCTCGACGTGATCCTGGACAAGGGAGTGGCCATCAA GGGCGACCTGATCATCTCTATCGCCGGCGTGGACCTGGTGTACCTGGATCTGAGAGTGCTGATCTC CAGCGTGGAAACCCTGGTGCAGGCCAAAGAGGGCAACCACAAGCCCATCACCAGCGAGCAGTTCGA CAAGCAGAAAGAGGAGCTGATGGACGCCACCGGCCAGCCCAGCAAGTGGACAAATCCTCTGGGCAG CGGCGCTCCCGGGTCAGGTGCCACGAATTTTTCGTTGTTGAAGCAAGCTGGGGATGTTGAAGAGAA CCCAGGGCCTGTGCAGCCCGTGTCCCAGGCCAACGGCAGAATCCACCTGGATCCCGATCAGGCCGA ACAGGGACTGGCCCAGCTCGTGATGACCGTGATCGAGCTGCTGCGGCAGATCGTGGAACGGCACGC CATGAGAAGAGTGGAAGGCGGCACCCTGACCGACGAGCAGATCGAGAATCTGGGAATCGCTCTGAT GAACCTGGAGGAGAAGATGGACGAGCTGAAAGAGGTGTTCGGACTGGACGCTGAGGATCTGAACAT CGACCTGGGCCCTCTGGGCAGCCTGCTGGGTGCCCCGGGATCTGGCGCAACAAATTTTAGTCTTTT AAAGCAGGCAGGAGACGTCGAGGAAAACCCTGGACCCGTGGCCGTGGAACACAACATGCAGAGCAG CACCATCGTGGACGTGCTGGAAAAGATCCTGGACAAGGGCGTCGTGATCGCCGGGGACATCACAGT GGGAATCGCCGACGTGGAACTGCTGACCATCAAGATCCGGCTGATCGTGGCCAGCGTGGACAAGGC CAAAGAAATCGGCATGGATTGGTGGGAGAACGACCCCTACCTGAGCAGCAAGGGCGCCAACAACAA GGCTCTGGAAGAGGAAAACAAGATGCTGCACGAGCGGCTGAAAACACTGGAAGAGAAGATCGAGAC AAAGCGCGGGGCACCTGGCTCGGGAGCGACCAACTTCTCATTACTCAAACAAGCCGGAGACGTTGA GGAGAATCCAGGCCCTGTGAGCACCGGCCCCAGCTTCAGCACCAAGGACAACACCCTGGAATACTT CGTGAAGGCCAGCAACAAGCACGGCTTTAGCCTCGACATCAGCCTGAACGTGAATGGGGCCGTGAT TAGCGGCACCATGATCAGCGCCAAAGAGTACTTCGACTACCTGAGCGAGACATTCGAAGAGGGCAG CGAAGTGGCCCAGGCCCTGTCTGAGCAGTTTAGCCTGGCTAGCGAGGCCTCCGAGTCTAATGGCGA AGCCGAGGCCCACTTCATCCACCTGAAGAACACCAAGATCTACTGCGGCGACAGCAAGAGCACCCC CAGCAAGGGCAAGATCTTCTGGCGCGGCAAGATCGCCGAGGTGGACGGATTCTTCCTGGGAAAAAT CAGCGACGCCAAGTCCACCAGCAAGAAGTCCAGCGGCGCTCCCGGGTCAGGTGCCACGAATTTTTC GTTGTTGAAGCAAGCTGGGGATGTTGAAGAGAACCCAGGGCCTGTGGTGTCCAAGGGCGAGGAACT GTTCACCGGCGTGGTGCCCATCCTGGTGGAACTGGATGGCGACGTGAACGGCCACAAGTTCAGCGT GTCCGGCGAGGGCGAAGGCGACGCCACATACGGAAAGCTGACCCTGAAGTTCATCTGCACCACCGG CAAGCTGCCCGTGCCTTGGCCTACCCTCGTGACCACACTGACCTACGGCGTGCAGTGCTTCGCCAG ATACCCCGACCACATGAAGCAGCACGATTTCTTCAAGAGCGCCATGCCCGAGGGCTACGTGCAGGA ACGGACCATCTTCTTCAAGGACGACGGCAACTACAAGACAAGAGCCGAAGTGAAGTTCGAGGGCGA CACCCTCGTGAACCGGATCGAGCTGAAGGGCATCGACTTCAAAGAGGATGGCAACATCCTGGGCCA CAAGCTGGAGTACAACTACAACAGCCACAAGGTGTACATCACCGCCGACAAGCAGAAAAACGGCAT CAAAGTGAACTTCAAGACCCGGCACAACATCGAGGACGGCAGCGTGCAGCTGGCCGACCACTACCA GCAGAACACCCCCATCGGAGATGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCACACAAAG CGCCCTGAGCAAGGACCCCAACGAGAAGCGGGACCACATGGTGCTGCTGGAATTTGTGACCGCCGC TGGCATCACCCTGGGCATGGACGAGCTGTACAAGTGAACTAGTTCGTTAACTAAACTTGTTTATTG CAGCTTATAATGGTTACAAATAAAGCAATAGCATCACAAATTTCACAAATAAAGCATTTTTTTCAC TGCATTCTAGTTGTGGTTTGTCCAAACTCATCAATGTATCTTATCATGTCTGGAATTGACTCAAAT GATGTCAATTAGTCTATCAGAAGCTCATCTGGTCTCCCTTCCGGGGGACAAGACATCCCTGTTTAA TATTTAAACAGCAGTGTTCCCAAACTGGGTTCTTATATCCCTTGCTCTGGTCAACCAGGTTGCAGG GTTTCCTGTCCTCACAGGAACGAAGTCCCTAAAGAAACAGTGGCAGCCAGGTTTAGCCCCGGAATT GACTGGATTCCTTTTTTAGGGCCCATTGGTATGGCTTTTTCCCCGTATCCCCCCAGGTGTCTGCAG GCTCAAAGAGCAGCGAGAAGCGTTCAGAGGAAAGCGATCCCGTGCCACCTTCCCCGTGCCCGGGCT GTCCCCGCACGCTGCCGGCTCGGGGATGCGGGGGGAGCGCCGGACCGGAGCGGAGCCCCGGGCGGC TCGCTGCTGCCCCCTAGCGGGGGAGGGACGTAATTACATCCCTGGGGGCTTTGGGGGGGGGCTGTC CCTGATATCTATAACAAGAAAATATATATATATAATAAGTTATCACGTAAGTAGAACATGAAATAACA ATATAATTATCGTATGAGTTAAATCTTAAAAGTCACGTAAAAGATAATCATGCGTCATTTTGACTC ACGCGGTCGTTATAGTTCAAAATCAGTGACACTTACCGCATTGACAAGCACGCCTCACGGGAGCTC CAAGCGGCGACTGAGATGTCCTAAATGCACAGCGACGGATTCGCGCTATTTAGAAAGAGAGAGCAA TATTTCAAGAATGCATGCGTCAATTTTACGCAGACTATCTTTCTAGGGTTAATCTAGCTGCATCAG GATCATATCGTCGGGTCTTTTTTCCGGCTCAGTCATCGCCCAAGCTGGCGCTATCTGGGCATCGGG GAGGAAGAAGCCCGTGCCTTTTCCCGCGAGGTTGAAGCGGCATGGAAAGAGTTTGCCGAGGATGAC TGCTGCTGCATTGACGTTGAGCGAAAACGCACGTTTACCATGATGATTCGGGAAGGTGTGGCCATG CACGCCTTTAACGGTGAACTGTTCGTTCAGGCCACCTGGGATACCAGTTCGTCGCGGCTTTTCCGG ACACAGTTCCGGATGGTCAGCCCGAAGCGCATCAGCAACCCGAACAATACCGGCGACAGCCGGAAC TGCCGTGCCGGTGTGCAGATTAATGACAGCGGTGCGGCGCTGGGATATTACGTCAGCGAGGACGGG TATCCTGGCTGGATGCCGCAGAAATGGACATGGATACCCCGTGAGTTACCCGGCGGGCGCGCTTGG

	CGTAATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATTCCACACAACATAC GAGCCGGAAGCATAAAGTGTAAAGCCTGGGGTGCCTAATGAGTGAGCTAACTCACATTAATTGCGT TGCGCTCACTGCCCGCTTTCCAGTCGGGAAACCTGTCGTGCCAGCTGCATTAATGAATCGGCCAAC GCGCGGGGAGAGGCGGTTTGCGTATTGGGCGCTCTTCCGCTTCCTCGCTCACTGACTCGCTGCGCT CGGTCGTTCGGCTGCGGCGAGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAAT CAGGGGATAACGCAGGAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGG CCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAA GTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCG TGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCG TGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGG GCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGT CCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGA GGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGGACAG TATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCG GCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAA AAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACTCAC GTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTCACCTAGATCCTTTTAAATTAAAAAT GAAGTTTTAAATCAATCTAAAGTATATATGAGTAAACTTGGTCTGACAGTTACCAATGCTTAATCA GTGAGGCACCTATCTCAGCGATCTGTCTATTTCGTTCATCCATAGTTGCCTGACTCCCCGTCGTGT AGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGACCCAC GCTCACCGGCTCCAGATTTATCAGCAATAAACCAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTC CTGCAACTTTATCCGCCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAGTAAGTAGTTCGC CAGTTAATAGTTTGCGCAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTCACGCTCGTCGTTTG GTATGGCTTCATTCAGCTCCGGTTCCCAACGATCAAGGCGAGTTACATGATCCCCCATGTTGTGCA AAAAAGCGGTTAGCTCCTTCGGTCCTCCGATCGTTGTCAGAAGTAAGTTGGCCGCAGTGTTATCAC TCATGGTTATGGCAGCACTGCATAATTCTCTTACTGTCATGCCATCCGTAAGATGCTTTTCTGTGA CTGGTGAGTACTCAACCAAGTCATTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGG CGTCAATACGGGATAATACCGCGCCACATAGCAGAACTTTAAAAGTGCTCATCATTGGAAAACGTT CTTCGGGGCGAAAACTCTCAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAACCCACTCGTG CACCCAACTGATCTTCAGCATCTTTTACTTTCACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGC AAAATGCCGCAAAAAAGGGAATAAGGGCGACACGGAAATGTTGAATACTCAT
PiggyBac transposon containing GvpJFGLK	ACTCTTCCTTTTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACATATT TGAATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCACCTAA ATTGTAAGCGTTAATATTTTGTTAAAATTCGCGTTAAATTTTTGTTAAATCAGCTCATTTTTTAAC CAATAGGCCGAAATCGGCAAAATCCCTTATAAATCAAAAGAATAGACCGAGATAGGGTTGAGTGTT GTTCCAGTTTGGAACAAGAGTCCACTATTAAAGAACGTGGACTCCAACGTCAAAGGGCGAAAAACC GTCTATCAGGGCGATGGCCCACTACGTGAACCATCACCCTAATCAAGTTTTTTGGGGTCGAGGTGC CGTAAAGCACTAAATCGGAACCCTAAAGGGAGCCCCCGATTTAGAGCTTGACGGGGAAAGCCGGCG AACGTGGCGAGAAAGGAAGGGAAGAAAGCGAAAGGAGCGGGCGCTAGGGCGCTGGCAAGTGTAGCG GTCACGCTGCGCGTAACCACCACACCCGCCGCGCTTAATGCGCCGCTACAGGGCGCGTCCCATTCG CCATTCAGGCTGCGCAACTGTTGGGAAGGGCGATCGGTGCGGGCCTCTTCGCTATTACGCCAGCTG GCGAAAGGGGGATGTGCTGCAAGGCGATTAAGTTGGGTAACGCCAGGGTTTTCCCAGTCACGACGT TGTAAAACGACGGCCAGTGAGCGCGCCTCGTTCATTCACGTTTTTGAACCCGTGGAGGACGGGCAG ACTCGCGGTGCAAATGTGTTTTACAGCGTGATGGAGCAGATGAAGATGCTCGACACGCTGCAGAAC ACGCAGCTAGATTAACCCTAGAAAGATAATCATATTGTGACGTACGTTAAAGATAATCATGTGTAA AATTGACGCATGTGTTTTATCGGTCTGTATATCGAGGTTTATTTATTAATTTGAATAGATATTAAG TTTTATTATATTTACACTTACATACTAATAATAAATTCAACAAACAATTTATTTATGTTTATTTAT TTATTAAAAAAAAACAAAAACTCAAAATTTCTTCTATAAAGTAACAAAACTTTTATGAGGGACAGCC CCCCCCCAAAGCCCCCAGGGATGTAATTACGTCCCTCCCCCGCTAGGGGGCAGCAGCGAGCCGCCC GGGGCTCCGCTCCGGTCCGGCGCTCCCCCCGCATCCCCGAGCCGGCAGCGTGCGGGGACAGCCCGG GCACGGGGAAGGTGGCACGGGATCGCTTTCCTCTGAACGCTTCTCGCTGCTCTTTGAGCCTGCAGA CACCTGGGGGGATACGGGGAAAAGGCCTCCACGGCCACTAGTTTCCCCGAAAAGTGCCACCTGAC GTCGGCAGTGAAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTA TAAGCTGCAATAAACAAGTTAACAACAACAATTGCATTCATTTTATGTTTCAGGTTCAGGGGGAGG TGTGGGAGGTTTTTTAAAGCAAGTAAAACCTCTACAAATGTGGTATGGCTGATTATGATCCTCTAG ACATATGCTGCAGTCACTTGTACAGCTCATCCATGCCCAGGGTGATGCCAGCGGCGGTCCGAAATT CCAGCAGCACCATGTGGTCCCGCTTCTCGTTGGGGTCCTTGCTCAGCACGCTCTGGGTGCTCAGGT AGTGGCTATCAGGCAGCAGCACGGGGCCATCTCCGATGGGGGTGTTCTGCTGGTAGTGGTCGGCCA

	GCTGCACGCTGCCATCTTCCACGTTGTGCCGGATCTTGAAGTTCACTTTGATGCCGTTTTTCTGCT TCACGGCCATGATGTAGATGTTGTGGCTGTTGAAGTTGTACTCCAGCTTGTGGCCCAGGATGTTGC CGTCCTCTTTGAAGTCCACGCCCTTCAGCTCGATCCGGTTCACGAGGGTGTCGCCCTCGAACTTCA CTTCGGCTCTGGTCTTGTAGGTGCCGTCGTCCTTGAAGAAGATGGTCCGTTCCTGCACGTAGCCCT CGGGCATGGCGCTCTTGAAGAAATCGTGCTGCTTCATGTGGTCGGGGTATCTGGCGAAGCACTGCA CGCCGTGAGACAGTGTGGTCACGAGGGTAGGCCAAGGCACGGGCAGCTTGCCGGTGGTGCAGATGA ACTTCAGGGTCAGCTTGCCATTTGTGGCGTCGCCTTCGCCCTCTCCCCGCACAGAGAACTTGTGGC CGTTCACGTCGCCATCCAGTTCCACCAGGATGGGCACCACGCCGGTGAACAGTTCCTCGCCCTTGG ACACCATGGTGAAGGGTACTGGATCCGAGCTCGGTACCTGCAGGCGTACCTTCTATAGTGTCACCT AAATGCGATCTGACGGTTCACTAAACGAGCTCTGCTTATATAGGCCTCCCACCGTACACGCCACCT CGACATACTCGAGTTTACTCCCTATCAGTGATAGAGAACGTATGAAGAGTTTACTCCCTATCAGTG ATAGAGAACGTATGCAGACTTTACTCCCTATCAGTGATAGAGAACGTATAAGGAGTTTACTCCCTA TCAGTGATAGAGAACGTATGACCAGTTTACTCCCTATCAGTGATAGAGAACGTATCTACAGTTTAC TCCCTATCAGTGATAGAGAACGTATATCCAGTTTACTCCCTATCAGTGATAGAGAACGTATGTCGA GGTAGGCGTGTACGGTGGGCGCCTATAAAAGCAGAGCTCGTTTAGTGAACCGTCAGATCGCCTGGA GCAATTCCACAACACTTTTGTCTTATACTTGGTACCTATGCATGCCACCATGGCCGTGGAACACAA CATGCAGAGCAGCACCATCGTGGACGTGCTGGAAAAGATCCTGGACAAGGGCGTCGTGATCGCCGG GGACATCACAGTGGGAATCGCCGACGTGGAACTGCTGACCATCAAGATCCGGCTGATCGTGGCCAG CGTGGACAAGGCCAAAGAAATCGGCATGGATTGGTGGGAGAACGACCCCTACCTGAGCAGCAAGGG CGCCAACAACAAGGCCCTGGAAGAGGAAAACAAGATGCTGCACGAGCGGCTGAAAACACTGGAAGA GAAGATCGAGACAAAGCGCGGTGCCCCGGGATCTGGCGCAACAAATTTTAGTCTTTTAAAGCAGGC AGGAGACGTCGAGGAAAACCCTGGACCCGTGAGCGAGACAAACGAGACAGGCATCTACATCTTCAG CGCCATCCAGACAGACAAGGATGAGGAATTCGGCGCCGTGGAAGTGGAAGGGACCAAGGCTGAGAC ATTCCTGATCCGGTATAAGGACGCCGCCATGGTGGCCGCCGAAGTGCCCATGAAGATCTACCACCC CAACCGGCAGAACCTGCTGATGCACCAGAATGCCGTGGCCGCCATCATGGACAAGAACGACACCGT GATCCCCATCAGCTTCGGCAACGTGTTCAAGAGCAAAGAGGACGTGAAGGTGCTCCTGGAAAACCT GTACCCCCAGTTCGAGAAGCTGTTCCCCGCCATCAAGGGAAAGATCGAAGTGGGCCTGAAGGTGAT CGGCAAGAAAGAGTGGCTCGAAAAGAAAGTGAACGAGAACCCCGAGCTGGAAAAAGTGTCCGCCAG CGTGAAGGGCAAGAGCGAGGCCGCTGGCTACTACGAGAGAATCCAGCTGGGCGGCATGGCCCAGAA GATGTTCACAAGCCTGCAGAAAGAAGTGAAAACCGACGTGTTCAGCCCCCTGGAAGAAGCCGCCGA GGCCGCCAAAGCCAATGAGCCTACAGGCGAAACAATGCTGCTGAACGCCAGCTTCCTGATCAACAG AGAGGATGAGGCCAAGTTCGACGAGAAAGTCAATGAGGCCCACGAGAACTGGAAGGATAAGGCCGA CTTCCACTACAGCGGCCCCTGGCCCGCCTACAACTTCGTGAACATCCGGCTGAAGGTGGAAGAGAA GGGGGCACCTGGCTCGGGAGCGACCAACTTCTCATTACTCAAACAAGCCGGAGACGTTGAGGAGAA TCCAGGCCCTGTGCTGCACAAGCTCGTGACCGCCCCCATCAACCTGGTCGTGAAGATCGGCGAGAA GGTGCAGGAAGAGGCCGACAAGCAGCTGTACGACCTGCCCACCATCCAGCAGAAGCTGATCCAGCT GCAGATGATGTTCGAGCTGGGCGAGATCCCCGAGGAAGCCTTCCAGGAAAAAGAGGACGAACTGCT GATGAGATACGAGATCGCCAAGCGGCGCGAGATTGAGCAGTGGGAAGAACTGACCCAGAAGCGGAA TGAGGAAAGCGGTGCCCCGGGATCTGGCGCAACAAATTTTAGTCTTTTAAAGCAGGCAGGAGACGT CGAGGAAAACCCTGGACCCGTGGGCGAGCTGCTGTACCTCTACGGCCTGATCCCCACCAAAGAGGC CGCTGCTATCGAGCCCTTCCCATTCTACAAGGGCTTCGACGGCGAGCACAGCCTGTACCCTATCGC CTTCGACCAAGTGACCGCCGTGGTGTTCAAGCTGGACGCCGACACCTACAGCGAGAAAGTGATCCA GGAAAAGATGGAACAGGACATGAGCTGGCTGCAGGAAAAGGCCTTCCACCACCACGAGACAGTGGC CGCCCTGTATGAGGAATTCACCATCATCCCCCTGAAGTTCTGCACCATCTATAAGGGAGAGGAATC CCTGCAGGCCGCCATCGAGATCAACAAAGAGAAGATCGAAAACTCCCTGACCCTGCTGCAGGGCAA CGAGGAATGGAACGTGAAGATCTACTGCGACGACACCGAGCTGAAGAAGGGCATCAGCGAGACAAA CGAGAGCGTGAAGGCCAAGAAGCAGGAAATCAGCCACCTGAGCCCCGGCAGACAGTTCTTCGAGAA GAAGAAGATTGACCAGCTCATCGAGAAAGAGCTGGAACTGCACAAGAACAAAGTGTGCGAGGAAAT CCACGACAAGCTGATTGAGCTGAGCCTCTACGACTCCGTGAAGAAGAACTGGTCCAAGGACGTGAC AGGCGCTGCCGAACAGATGGCCTGGAACAGCGTGTTCCTGCTGCCCAGCCTGCAGATCACCAAGTT CGTGAACGAGATCGAGGAACTCCAGCAGCGGCTGGAGAACAAGGGATGGAAGTTCGAAGTGACCGG CCCCTGGCCTCCCTACCACTTCAGCAGCTTTGCCGGGGCACCTGGCTCGGGAGCGACCAACTTCTC ATTACTCAAACAAGCCGGAGACGTTGAGGAGAATCCAGGCCCTGTGCAGCCCGTGTCCCAGGCCAA CGGCAGAATCCACCTGGATCCCGATCAGGCCGAACAGGGACTGGCCCAGCTCGTGATGACCGTGAT CGAGCTGCTGCGGCAGATCGTGGAACGGCACGCCATGAGAAGAGTGGAAGGCGGCACCCTGACCGA CGAGCAGATCGAGAATCTGGGAATCGCCCTGATGAACCTGGAAGAGAAGATGGACGAGCTGAAAGA GGTGTTCGGACTGGACGCCGAGGACCTGAACATCGACCTGGGCCCTCTGGGCAGCCTGCTGTGAAC TAGTTCGATACCGTCGACCGTTAACTAAACTTGTTTATTGCAGCTTATAATGGTTACAAATAAAGC

	AATAGCATCACAAATTTCACAAATAAAGCATTTTTTTCACTGCATTCTAGTTGTGGTTTGTCCAAA CTCATCAATGTATCTTATCATGTCTGGAATTGACTCAAATGATGTCAATTAGTCTATCAGAAGCTC ATCTGGTCTCCCTTCCGGGGGACAAGACATCCCTGTTTAATATTTAAACAGCAGTGTTCCCAAACT GGGTTCTTATATCCCTTGCTCTGGTCAACCAGGTTGCAGGGTTTCCTGTCCTCACAGGAACGAAGT CCCTAAAGAAACAGTGGCAGCCAGGTTTAGCCCCGGAATTGACTGGATTCCTTTTTTAGGGCCCAT TGGTATGGCTTTTTCCCCGTATCCCCCCAGGTGTCTGCAGGCTCAAAGAGCAGCGAGAAGCGTTCA GAGGAAAGCGATCCCGTGCCACCTTCCCCGTGCCCGGGCTGTCCCCGCACGCTGCCGGCTCGGGGA TGCGGGGGGAGCGCCGGACCGGAGCGGAGCCCCGGGCGGCTCGCTGCTGCCCCCTAGCGGGGGAGG GACGTAATTACATCCCTGGGGGCTTTGGGGGGGGGCTGTCCCTGATATCTATAACAAGAAAATATA TATATAATAAGTTATCACGTAAGTAGAACATGAAATAACAATATAATTATCGTATGAGTTAAATCT TAAAAGTCACGTAAAAGATAATCATGCGTCATTTTGACTCACGCGGTCGTTATAGTTCAAAATCAG TGACACTTACCGCATTGACAAGCACGCCTCACGGGAGCTCCAAGCGGCGACTGAGATGTCCTAAAT GCACAGCGACGGATTCGCGCTATTTAGAAAGAGAGAGCAATATTTCAAGAATGCATGCGTCAATTT TACGCAGACTATCTTTCTAGGGTTAATCTAGCTGCATCAGGATCATATCGTCGGGTCTTTTTTCCG GCTCAGTCATCGCCCAAGCTGGCGCTATCTGGGCATCGGGGAGGAAGAAGCCCGTGCCTTTTCCCG CGAGGTTGAAGCGGCATGGAAAGAGTTTGCCGAGGATGACTGCTGCTGCATTGACGTTGAGCGAAA ACGCACGTTTACCATGATGATTCGGGAAGGTGTGGCCATGCACGCCTTTAACGGTGAACTGTTCGT TCAGGCCACCTGGGATACCAGTTCGTCGCGGCTTTTCCGGACACAGTTCCGGATGGTCAGCCCGAA GCGCATCAGCAACCCGAACAATACCGGCGACAGCCGGAACTGCCGTGCCGGTGTGCAGATTAATGA CAGCGGTGCGGCGCTGGGATATTACGTCAGCGAGGACGGGTATCCTGGCTGGATGCCGCAGAAATG GACATGGATACCCCGTGAGTTACCCGGCGGGCGCGCTTGGCGTAATCATGGTCATAGCTGTTTCCT GTGTGAAATTGTTATCCGCTCACAATTCCACACAACATACGAGCCGGAAGCATAAAGTGTAAAGCC TGGGGTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCCGCTTTCCAGTCG GGAAACCTGTCGTGCCAGCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCGTATT GGGCGCTCTTCCGCTTCCTCGCTCACTGACTCGCTGCGCTCGGTCGTTCGGCTGCGGCGAGCGGTA TCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGAACATG TGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGG CTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGA CTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCG CTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGT AGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAG CCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCG CCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTC TTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCTCTGCTGAAG CCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGT GGTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATC TTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACTCACGTTAAGGGATTTTGGTCATGAGATTA TCAAAAAGGATCTTCACCTAGATCCTTTTAAATTAAAAATGAAGTTTTAAATCAATCTAAAGTATA TATGAGTAAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGT CTATTTCGTTCATCCATAGTTGCCTGACTCCCCGTCGTGTAGATAACTACGATACGGGAGGGCTTA CCATCTGGCCCCAGTGCTGCAATGATACCGCGAGACCCACGCTCACCGGCTCCAGATTTATCAGCA ATAAACCAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTCCTGCAACTTTATCCGCCTCCATCCAG TCTATTAATTGTTGCCGGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTGCGCAACGTTGTT GCCATTGCTACAGGCATCGTGGTGTCACGCTCGTCGTTTGGTATGGCTTCATTCAGCTCCGGTTCC CAACGATCAAGGCGAGTTACATGATCCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGTCCT CCGATCGTTGTCAGAAGTAAGTTGGCCGCAGTGTTATCACTCATGGTTATGGCAGCACTGCATAAT TCTCTTACTGTCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTC TGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGATAATACCGCGCCA CATAGCAGAACTTTAAAAGTGCTCATCATTGGAAAACGTTCTTCGGGGCGAAAACTCTCAAGGATC TTACCGCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGCACCCAACTGATCTTCAGCATCTTTT ACTTTCACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAAGGGAATAAGG GCGACACGGAAATGTTGAATACTCAT

Table S5 – mARG gene cassette in the piggyBac backbone.

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